Design of a Bioreactor to Cyclically Strain Tissue Engineered Blood Vessels

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Authorship

The final version of this paper was written in equal parts by all group members. All group members were involved in editing all sections of the paper.

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Abstract

Cyclic mechanical loading improves strength and extracellular matrix (ECM) synthesis in tissue engineered blood vessels (TEBV). The goal of this project was to design a device to impart cyclic circumferential stretch on cell-derived TEBV rings cultured on flexible silicone tubing. Our device cyclically displaces water (75 µl volume) to inflate tubing at a frequency of 1 Hz. During static inflation tests, the tubing diameter increased by 10±1.6%. TEBV rings were loaded onto silicone tubing and subjected to mechanical conditioning for 3-7 days. Our device conditioned samples in an incubator at 10% distension at a frequency of 1 Hz. Static and conditioned tissues remained viable and uncontaminated and exhibited high cell densities and increased thicknesses from 3 to 7 days.

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Chapter 1 – Introduction

Most current technologies used to develop small diameter tissue engineered blood vessels (TEBVs) do not meet the clinical needs for a mechanically sound graft without extensive time and conditioning in culture. Ideally, autologous tissue engineered vessels or grafts would be cultured *in vitro* then applied *in vivo*. This concept has motivated researchers to study engineered vessels, and is the basis for this project. There are currently a variety of methods for the development of small diameter vasculature; however, many current technologies present a number of concerns and limitations.

Originally, engineers developed synthetic grafts made from materials such as Dacron and polytetrafluoroethylene (Zacharias et. al., 1987) in hopes of creating a mechanically sound replacement for human tissue. These grafts however are limited to vessels with larger diameters. In vessels smaller than 6 mm in inner diameter, the synthetic grafts often failed due to thrombosis (Atala et. al., 2008). Thrombosis is the formation of blood clots that occlude the vessel. The synthetic materials used in the vascular replacement procedures often initiated an immune response. This response would result in high levels of inflammation and failure of the graft (O'Donnell et. al., 1984). In an attempt to improve the biocompatibility of these materials, scientists have researched biomaterials that, when combined with autologous tissues, could result in biointegration of the graft (Mitchell et. al., 2003). The current standard for vessel replacement is the CABG, or coronary artery bypass graft, method. The CABG procedure requires a type of graft called an autologous graft. These grafts are sections of blood vessels less than 6 mm inner diameter, such as the saphenous vein, which are harvested from the patient. The graft is then implanted into the coronary circulation system to redirect flow past the damaged or occluded vessels. This approach is viable for ten to twelve years; any longer could result in narrowing of the vessel, resulting in a need for a second surgery. (L'Heureux, 2003; Goldman et. al., 2004)

Tissue engineered blood vessels are a potential solution to the complications that arise from the use of the previously mentioned vascular repair approaches. TEBVs are constructed by assembling cells taken from the patient into tubular constructs. These constructs can then be implanted into the patient, and in theory, should have mechanical properties similar to healthy tissue. Two-dimensional models, such as skin grafts, have been successfully created; three dimensional models are currently limited due to the samples' poor structural integrity and low mechanical strength. In order to be deemed successful, TEBVs

should be able to withstand physiological loads from blood pressure and fluid dynamics within the body. Additionally, the samples must be able to resist tearing, especially during suturing and handling. (Mitchell et. al., 2003)

In 2003, a tissue engineering technique was patented that used a cell sheet-based method that cultured fibroblasts and smooth muscle cells (SMCs) around a cylindrical mandrel. The result was a fully autologous graft that was capable of withstanding mechanical loads greater than that of blood pressure (over 2200 mm Hg) and was tear-resistant. Since the graft was composed of living cells, it was self-renewing resulting in an increased healing potential. Additionally, the graft was completely biological and could be remodeled by the body during the healing process. Remodeling is a response of the cells to the mechanical manipulations enacted by the surrounding environment. (L'Heureux, 2003)

Even though the tissue-based grafts greatly improved on past technologies, concerns and limitations remain. This approach used SMCs to improve the graft's strength. However, while the addition of these cells increased the strength they limited the graft's ability to expand and contract. Postoperative studies showed that while implanted, *in vivo* remodeling of the graft resulted in higher levels of elastin (L'Heureux, 2003). Elastin is a protein that acts like a rubber band as it contracts the vessel. In order to overcome the stiffness resulting from high levels of SMCs and collagen, it is vital to have enough elastin so that the vessel can return to the original diameter during pulsatile and pressurized loads. The elastin network also contributes to the long-term success of tissue engineered grafts. If a graft is too stiff, there will be a difference between the natural tissue and the graft causing the fluid dynamics to change, thus impacting the stream of fluids as they pass through the engineered vessel. The increased failure rate due to compliance differentials is seen with synthetic materials as well (Mitchell et. al., 2003). An additional concern with the application of SMCs as a means of structural support is that it remains difficult to regulate their proliferation once implanted. If the cells proliferate excessively, the graft can actually occlude itself resulting in failure. (L'Heureux, 2003)

Collagen networks are the natural means of vasculature support and can be induced in TEBVs in appropriate culture environments. The use of collagen gel-based grafts has been studied as a potential means for the reduction of the dependency on SMCs. The collagen gel is easier to control and improves the strength of the graft. Similar to the elastin networks, remodeling enzymes promote the synthesis of these networks. (Mauck et. al., 2009)

In a 2009 study which compared the effectiveness of TEBVs to that of autologous grafts of saphenous and mammary arteries, cyclic strains were proven to increase the production of collagen networks, which is indicative of higher tensile strength. This study specifically examined burst pressure, suture retention strength, and compliance of mechanically conditioned TEBVs in comparison to native saphenous veins. (Konig et. al., 2009)

The TEBVs were superior to the autologous grafts in burst pressure and suture retention, but the compliance ratios of the TEBVs were much lower. The long-term study showed that the cells appeared to react to their environment and maintain mechanical homeostasis. After six months of implantation, the TEBV compliance ratio increased by over 100%, whereas the native tissue samples remained constant. This increase in compliance validates the notion that a tissue sample exposed to mechanical manipulation is capable of remodeling and improving its mechanical properties. (Konig et. al., 2009) In this instance, the body functions as the ideal bioreactor, conditioning the TEBVs through physiological cardiac loading while maintaining cell viability. This response to physiological mechanical environments provides insight into how mechanical conditioning during culture could result in an improved graft. If cells respond to *in vivo* mechanical manipulation, they should also respond to *in vitro* loading assuming appropriate culturing techniques are maintained. These outcomes acted as the inspiration for the development of the *in vitro* mechanical loading model presented in this paper.

The Rolle Lab at Worcester Polytechnic Institute (WPI) uses tissue engineered blood vessel rings to model the mechanical and physiological properties of complete blood vessels. Rat aortic smooth muscle cells (SMCs) are grown in agarose molds over 8 days to form completely cell-derived, three-dimensional rings with an inner diameter of 2, 4, or 6 mm. It has also been shown that rings cultured in close proximity for an extended period of time could fuse to form a single cohesive tube, demonstrating possible future applications for *in vivo* studies. Furthermore, the rings' relatively small size and short production time allowed us to experiment with greater sample sizes. (Gwyther et. al., 2011)

The goal of this project was to design a bioreactor that would impart mechanical conditioning on 2 mm inner diameter tissue rings from the Rolle Lab. The bioreactor also had to be capable of maintaining basic cell culture conditions. It was then our responsibility to conduct an in-depth literature review in order to identify the limitations of bioreactors used to mechanically condition tissue constructs. As we developed an understanding of the problem, we began compiling a list of objectives and constraints. Using a collection of client interview data, we applied these objectives and constraints and created a list

of functions and means for our device. These means were then analyzed based on client input, material biocompatibility and manufacturability, and projected expenses, and several design alternatives were created. The designs were further scrutinized based on the plausibility of component interfaces and the availability of materials and manufacturing tools. We were able to determine our final design, which we prototyped and evaluated through bench top testing and culture experiments.

Additionally, sections of this paper examine the effects our device may have on the environment and the economy. Finally, future recommendations were made so that future teams could learn from our results and conclusions and further develop the device.

Chapter 2 – Literature Review

2.1 Mechanical Conditioning

2.1.1 Definition

In order for tissue engineered blood vessels (TEBVs) to function successfully, they must have high strength and endurance (Butler et. al., 2009) capable of withstanding the natural forces of the human body. These forces include shear stresses due to blood flow, vessel wall expansion and contraction due to pressure, as well as longitudinal tension along the vessel (Isenberg et. al., 2006). A diagram of these forces is pictured in **Figure 1**. The goal of mechanical conditioning of tissue engineered blood vessels is to mimic these natural *in vivo* forces while TEBVs are growing *in vitro*.

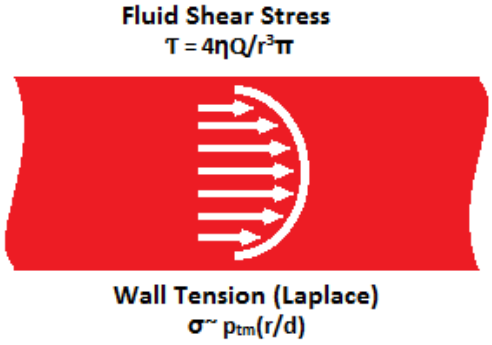


Figure 1. Forces experienced within natural blood vessels

Mechanical conditioning is a widely used method for treating engineered tissue constructs to enhance their physical and mechanical properties. Techniques for creating artificial tissues have used various cell types, such as smooth muscle and endothelial, as well as differing culture conditions to create grafts that emulate native tissue (Isenberg et. al, 2006; Isenberg et. al., 2003).

Cells are affected by an immense variety of chemical and mechanical stimuli. Bone and muscle cells in particular are known to respond to these mechanical stresses in a positive way (Neidlinger-Wilke et. al., 1995), constantly remodeling based on the stresses they are exposed to. Mechanical stretching of smooth muscle cells (SMCs) cultured in collagen-based tissue equivalents has been shown to have immense effects on SMC orientation (Dartsch et. al., 1986; Kanda et. al., 1993; Liu, 1998), ECM deposition (Chiquet et. al., 1996; Kolpakov et. al., 1995; Kim et. al., 1999; Niklason et. al., 1999; Kulik et.

al., 1993), and release of cellular growth factors (Cheng et. al., 1997; Sudhir et. al., 2001). Liu's 1998 study found that under circumferential strain, rat blood vessel SMCs arrange themselves in the circumferential direction. Kim (1999) found that cyclic mechanical strain (7% distension at 1 Hz for 4 days) applied to smooth muscle tissue grafted onto polymeric scaffolds increased SMC proliferation and collagen and elastin expression. This resulted in enhanced mechanical properties and long term tissue organization. Cheng (1997) found that mechanical strain influences fibroblast growth factor-2 (FGF-2) release from human SMCs. As the amplitude of deformation rose, more FGF-2 was released in response; from 4% amplitude (90 cycles at 1 Hz) releasing 0.1±0.1% FGF-2 to 14% (90 cycles at 1 Hz) releasing 5.7±0.5% FGF-2.

In order for engineered musculoskeletal, vascular, and cardiac tissues to function successfully without failure *in vivo*, mechanical modulation must be considered. Ideally, a combination of mechanical and biochemical factors will lead to engineered constructs whose functionality and mechanical properties closely mimic those of native tissue. Perfecting mechanical conditioning techniques is of the utmost importance for cardiovascular tissue engineering before *in vivo* applications can be seriously considered (Rubbens et. al., 2009; Butler et. al., 2009). Mechanical forces are becoming as important as growth factors or cytokines in the overall growth and development of TEBVs. (Freed et. al., 2006)

2.1.2 Impact on TEBV Structure and Properties

1. Benefits of Mechanical Conditioning

Naturally occurring vascular SMCs are not exposed directly to shear stress by normal blood flow, they are primarily affected by pulsatile distension during the cardiac cycle (Isenberg et. al., 2003). Due to this, tissue engineered blood vessels are primarily conditioned based on the dominant stress encountered *in vivo,* cyclic mechanical distension. Studies have shown that cyclic mechanical distension strengthens engineered vessels (Niklason et. al., 1999; Freed et. al., 2006; Isenberg et. al., 2006; Syedain et. al., 2008; Seliktar et. al., 2000; Huynh et. al., 2010) and promotes collagen production and cell alignment (Niklason et. al., 1999; Freed et. al., 2006; Isenberg et. al., 2003; Schutte et. al., 2010; Seliktar et. al., 2000). It also has enormous beneficial effects on SMC phenotype, ECM deposition, growth factor release, and vascular tone (Isenberg et. al., 2003). Biomechanical factors influence tissue growth (Isenberg et. al., 2003; Niklason et. al., 1999; Syedain et. al., 2008), development (Guilak et. al., 1997; Niklason et. al., 1999), maintenance (Butler et. al., 2009; Syedain et. al., 2008), degeneration (Butler et. al., 2009; Fujiwara et. al., 2003), and repair (Cheng et. al., 1997; Fujiwara et. al., 2003).

Cells receiving mechanical stimuli will align themselves in the direction of the stretch or flow to combat vessel weakness and increase cell length. Weakness could occur when cells are aligned in a random pattern within the wall of a vessel versus aligning along the direction of blood flow. Collagen and elastin fibrils will similarly align in a more compact manner in the direction of tension, increasing vessel strength and stiffness (Isenberg et. al., 2003; Tower et. al., 2002). TEBVs also respond to cyclic distension by producing more collagen and elastin, changing the composition of the extracellular matrix and further strengthening and stiffening vessel walls. Mechanical stretching has been shown to promote the expression of type I and III collagens, fibronectin, and tenascin-C in cultured ligament fibroblasts (Sahoo et. al., 2007).

Mechanical properties can be engineered to match those of natural blood vessels. The burst pressure of a saphenous vein typically used in coronary artery bypass grafts in patients with heart disease is 1680 ± 307 mmHg. (Konig et. al., 2009) A tissue engineered blood vessel cultured for 7 weeks with the same diameter and length as the tested saphenous vein had a burst pressure of 2594 ± 501 mmHg (Konig et. al., 2009). Vascular compliance ($\frac{\Delta V}{\Delta P}$; ΔV=change in volume, ΔP=change in pressure), stiffness ($\frac{F}{\delta}$; F=force applied, δ=displacement produced by force), tensile strength (calculated based on uniaxial tensile testing, ultimate tensile stress, UTS, at failure), and burst pressure ($P = \frac{(2t*S)}{O}$; t=wall thickness (in), S=tensile strength of blood vessel (PSI), O=outer diameter (in)) can be altered and potentially controlled for based on the frequency and force used during conditioning.

Compliance and stiffness are important properties contributing to overall TEBV health. Compliance refers to the ability of a blood vessel to recoil to its original shape after deformation by force. Stiffness is the blood vessels resistance to this deformation. Tensile strength is the maximum stress that a blood vessel can handle in a 2-D plane before failure. Burst pressure denotes the maximum stress that a blood vessel can handle in a 3-D plane before bursting during pressurization. Vascular stiffness correlates with increased cardiovascular risk (DeLoach et. al., 2008). By controlling these parameters, tissue engineered blood vessel properties can be catered to the needs of individual patients by altering the specific vascular type and size and decreasing susceptibility to cardiovascular disease.

Increased ECM synthesis is a positive effect of biomechanical conditioning (Mauck et. al., 2000; Freed et. al., 2006; Buschmann et. al., 1995; Ku et. al., 2006; Niklason et. al., 1999; Seliktar et. al., 2000; Syedain et. al., 2009). Mauck (2000) studied dynamically loaded tissue engineered articular cartilage seeded on

agarose gels. Tissue disks were dynamically compressed at 10% strain at a frequency of 1 Hz; at a duty cycle of 1 hour on, 1 hour off per day for 5 days a week and 4 weeks total. Mauck (2000) found a sixfold increase in equilibrium aggregate modulus over controls and matrix elaboration due to heightened ECM synthesis over time for conditioned samples.

Syedain (2009) created tissue engineered heart valves (TEHVs) by entrapping dermal fibroblasts into molded fibrin gels left to statically incubate for 2 weeks. These TEHVs were then strained incrementally at 5% (1st week), 10% (2nd week), and 15% (3rd week) over a 3 week period in a custom-built bioreactor at a frequency of 0.5 Hz. Syedain found that after cyclic stretching, collagen and cellular alignment was primarily circumferential, which correlates with native TEHVs. Collagen also appeared more organized into mature bundles at a density 86% greater than static TEHVs. Tensile strength and elastic modulus were also improved by over 97% and 77%, respectively, compared to static TEHVs.

Mechanical properties of tissue engineered constructs (such as tensile strength, elastic modulus, and vessel stiffness) have been improved by mechanical conditioning (Hahn et. al., 2007; Diamantouros et. al., 2009; Freed et. al., 2006; Fujiwara et. al., 2003; Gimbronejr et. al., 1999; Isenberg et. al., 2006; Isenberg et. al., 2003; Konig et. al., 2009; Ku et. al., 2006; Niklason et. al., 1999; Schutte et. al., 2010). Hahn (2007) used mouse smooth muscle progenitor cells within poly(ethyleneglycol)-based hydrogels with adhesive ligands and a collagenase degradable sequence to create 3mm inner diameter tissue engineered vascular grafts. These grafts were subjected to 7 weeks of mechanical conditioning under pulsatile flow at 2 mL/sec. Results showed significantly higher collagen levels (resulting in higher tensile strength) and improved elastic moduli compared to static samples.

Overall, TEBVs show much promise as alternatives to autografting for vascular replacements based on their burst strength, suturability, and mechanical similarities to native vessels.

2. Drawbacks of Mechanical Conditioning

Mechanical conditioning in culture is a difficult challenge. Systems have been developed which apply mechanical forces via piston/compression systems, substrate bending, hydrodynamic compression and fluid shear (Dobson et. al., 2006). Problems exist with these systems, namely, long term sterility and cells receiving adequate nutrients through the thickness of the vessel. In systems that use scaffolded tissue constructs, the scaffold takes on a portion of the mechanical load. Therefore, the tissue in culture

does not receive the full impact of the mechanical conditioning and as a result the production of ECM is hindered. (Dobson et. al., 2006)

Studies have shown that excessive cyclic stretch is detrimental to cells (Fujiwara et. al., 2003; Gassman et. al., 2010). Fujiwara (2003) modeled this phenomenon using endothelial lung tissue. Lung tissue naturally stretches by an estimated 5% during normal breathing *in vivo*, but anything higher than 17-22% may damage the endothelial cells of the lungs. The key is to condition cells at an "ordinary", tolerable percentage that won't over-stimulate or harm them (Fujiwara et. al., 2003). Despite these limitations, mechanical conditioning remains a widely used method to prepare tissue engineered constructs for the rigorous environment of the human body as it improves ECM synthesis, cellular alignment, and mechanical properties.

2.1.3 Types and Studies Demonstrating Mechanical Conditioning Techniques

Several different mechanical conditioning techniques have been shown to alter the mechanical and physiological characteristics of tissues. These techniques include magnetic stress, dynamic compressive loading, flow-mediated shear stress, continuous mechanical tension, stretching on membranes, and cyclic mechanical distension. Different tissue types require different mechanical stimuli to produce tissues with specific characteristics. For instance, mechanical conditioning that focuses on applied compressive forces would enhance a tissue's compressive strength, while mechanical tension or stretching would improve elasticity and compliance. Cyclic mechanical distension is the type of conditioning the team chose to use for the purposes of our project.

1. *Cyclic Mechanical Distension*

A study conducted in 2000 by Seliktar cyclically strained collagen-gel blood vessel constructs composed of adult rat aortic SMCs mounted on etched silicone sleeves (coated in type I collagen and chitosan). The sleeves and constructs were sutured into in a sterile, air-tight bioreactor where media was added and 0.2 µm filters served as vents for gas exchange. The tissue constructs were cycled within an incubator at a frequency of 1 Hz and 10% cyclic strain for 4 and 8 days. The silicone sleeve was distended using a regulated compressed air supply and a solenoid valve located outside the incubator.

The vessels showed increased contraction and mechanical strength when compared to statically grown control constructs. The vessels conditioned for 8 days showed significant increases in ultimate tensile stress (the maximum load divided by the initial cross-sectional area of a sample) and material modulus

(stress divided by strain). Morphologically, the mechanically conditioned vessels showed higher circumferential orientation of the vessel cells. (Seliktar et. al., 2000)

Another study focused on the effects of applied stretching on rabbit pulmonary arteries. They found that after four days of stretching, the amount of pro-collagen type I-positive cells grew, along with higher levels of protein synthesis within the smooth muscle cells (SMCs) and cell replication within the construct. (Kolpakov et. al., 1995) A similar study by Liu in 1998 used rat blood vessels. They discovered that continuous tensile strain on the vessels tested in 10 day increments over a period of 30 days resulted in better alignment of the SMCs. (Liu, 1998)

Brett Isenberg and Robert Tranquillo (2003) studied the effects of long-term cyclic distension on collagen-based media equivalents (denatured, cross-linked type I collagen) consisting of adult rat aortic SMCs. The SMCs were grown and incubated on Teflon rod mandrels in standard cell culture medium, changed once per week. The tissue constructs themselves were 1-3 mm in length, 0.2-0.3 mm thick, and 8 mm in inner diameter. The tissue constructs were conditioned on distensible latex mandrels inflated by pressurized air within an incubator

The collagen-based media equivalents (MEs) were strained incrementally to the target percentage over 3 days and remained at that frequency (0.5 Hz) and strain level (12.5%) for 5 weeks. The tissues were tested for various mechanical properties and collagen content. They found that mechanical conditioning for 2 weeks yielded little change in elastic modulus (E) or ultimate tensile strength (UTS), but conditioning for 5 weeks resulted in significant increases in both. Isenberg and Tranquillo found that cell age has no influence on response to stimulation and that 2.5% and 5% strain values are best for high UTS. They also found that a 10% strain value during mechanical conditioning is best for obtaining a high elastic modulus and higher elastin content within samples. This study failed to confirm whether or not an increase in cell number, collagen content, or fibril alignment was present in their tissue constructs as many other studies have claimed to have found. (Isenberg et. al., 2003)

Niklason (1999) used pulsatile radial flow to cyclically distend silicone tubing loaded with PGA scaffolds seeded with neonatal SMCs at 2.75 Hz and 5% strain for 8 weeks. Niklason argued that pulsatile flow conditions (165 beats per minute and 5% radial distension) would more accurately represent conditions seen *in vivo,* and thus produce vessels better suited for eventual *in vivo* use. This study resulted in SMCs with an increase in modulus, collagen and elastin deposition, suture retention, and calponin and myosin

heavy chain (contractile components). Vessel wall thickness was also increased with longer culture time and pulsatile culture conditions. After treating the conditioned SMCs with prostaglandin $F_{2\alpha}$, the structure physically contracted as a natural blood vessel should. **Table 1** summarizes the different characteristics measured in Niklason's study. (Niklason et. al., 1999)

Table 1. Characteristics of engineered vessels in Niklason's 1999 study.

Vessel type*	Wall thickness (cm)	Collagen (% dry weight)	Suture retention (g)	SMC density (10^8 cells/ml)
5 P	0.019 ± 0.006 ($n = 4$)	29 ± 6 ($n = 4$)	40 ± 16 ($n = 4$) ($P < 0.001$)	0.40 ± 0.16 ($n = 4$)
5 NP	0.009 ($n = 1$)	†	2.7 ± 1.2 ($n = 3$)	†
8 P	0.038 ± 0.004 ($n = 5$)	50 ± 5 ($n = 6$) ($P < 0.005$)	91 ± 26 ($n = 6$) ($P < 0.005$)	0.93 ± 0.37 ($n = 5$)
8 NP	0.023 ± 0.004 ($n = 3$)	35 ± 3 ($n = 4$)	22 ± 8 ($n = 6$)	1.19 ± 0.14 ($n = 3$)
Native	0.029 (*18*)	45 ± 9‡	273 ± 31‡	2.87 ± 0.14§

*P, pulsed; NP, nonpulsed; number represents weeks. †Data insufficient. ‡Measured from bovine muscular arteries stripped of adventitia. §Measured from bovine arteries by fluorometric assay.

2. Other Types of Mechanical Conditioning

Magnetic stress, dynamic compressive loading, flow-mediated shear stress, continuous mechanical tension, and stretching on membranes are all effective methods commonly used in the tissue engineering community to alter the physical and mechanical properties of tissue engineered constructs.

Jon Dobson (2006) developed and prototyped a unique method to mechanically condition stem cells *in vitro* using biocompatible magnetic nanoparticles (MNPs) and ion channels present within the stem cells themselves. Dobson's device applies stress to the cell membrane using forces applied directly to magnetic micro- and nanoparticles attached to the cell membrane via surface receptors. Quantitative results of this bioreactor system's effects on tissue mechanical properties do not yet exist, but are currently being modeled *in vitro*. (Dobson et. al., 2006)

Dynamic compressive loading is another effective means of conditioning cell constructs. Ballyns (2010) investigated the effects of dynamic compression loading on engineered bovine meniscal fibrochondrocytes seeded and crosslinked in alginate hydrogels and shaped as menisci. A custom bioreactor applied sinusoidal displacement in alternating one hour increments 3 times a week for 6 weeks total. After 2 weeks, collagen content in conditioned samples increased 1.8 fold, compressive

modulus by 1.8-2.3 fold, and ECM content by 2-3.2 fold compared to static samples. (Ballyns et. al., 2010)

Flow-mediated shear stress is another method. *In vitro* models are limited in nutrient delivery due to a lack of blood supply. All nutrient/media delivery must occur through diffusion. Dynamic media flow within or around tissue-engineered constructs offer the best solution to enhance media delivery and waste exchange. They also simultaneously deliver flow-mediated shear stresses to cells seeded within the constructs, improving cell strength and structure (Butler et. al., 2009). Fluid shear stress at physiological (rest) conditions is estimated to be 1-50 dyn/cm^2 in human arterial endothelial cells (Fujiwara et. al., 2003).

A recent study has demonstrated that placing axons under continuous mechanical tension increases axon growth rates up to 1cm/day. This was accomplished by using a microstepper motor system to incrementally separate two neural membranes, allowing for spontaneous growth of axons from one membrane to another. This research is useful in the repair of nerve injuries using transplantable neurons with 10cm long axons for central nervous system repair. (Butler et. al., 2009; Pfister et. al., 2006; Smith et. al., 2001)

Research was conducted concerning the effects of mechanical stretching on collagen synthesis by mesenchymal stem cells and aortic valve interstitial cells. Ku (2006) found that at 14% stretch there was a significant increase in collagen synthesis in both types of cells, directly affecting the rate of ECM production and valve strength. Collagen synthesis was dependent on the degree and duration of stretch in aortic valve tissue. (Ku et. al., 2006) In another study, rat vascular smooth muscle cells were subjected to cyclic strain induced by a vacuum below the culture plates the cells were grown on. Cyclic strain caused an increase in total cell count by 40% compared to control tests. (Wilson et. al., 1993)

2.1.4 Conclusions

The ultimate goal of blood vessel engineering is to create a nonthrombogenic, nonimmunogenic, suturable tissue that is able to withstand arterial pressures, is compliant, and can be remodeled in response to injury (Schutte et. al., 2010). Mechanical stimulation improves the overall structure and function of native and tissue engineered vasculature alike; this is imperative for achieving these goals. Biomechanical forces have been shown to enhance the physical characteristics of TEBVs. Mechanical cues cause change in cell morphology, physiology, biochemistry, and gene expression, all of which

contribute to overall tissue function *in vivo*. (Butler et. al., 2009; Diamantouros et. al., 2009; Freed et. al., 2006; Isenberg et. al., 2003; Niklason et. al., 1999; Seliktar et. al., 2000; Syedain et. al., 2009) Cyclic mechanical distension of tissue engineered constructs has been shown to increase contraction and mechanical strength (Seliktar et. al., 2000; Isenberg et. al., 2003), increase circumferential cellular alignment along the vessel length (Seliktar et. al., 2000; Liu, 1998), and increase ECM synthesis and cell growth (Kolpakov et. al., 1995).

Mechanical conditioning has become a major goal of recent tissue engineering efforts and has led to a shift in some of the primary bioreactor design goals. Bioreactors have evolved from acting solely as culturing devices to culturing while simultaneously conditioning tissue constructs.

2.2 Bioreactors

Bioreactors are biomimetic laboratory devices that regulate a number of environmental factors in order to create an optimal platform for biological activity. In tissue culturing and engineering, a regulated environment is crucial in order to promote cell growth and tissue structure and mechanics; bioreactors can be designed to control variables including pH, temperature, hydration, nutrition, and waste regulation and removal. Many of these conditions may be provided by an incubator. In the past decade, the focus of these bioreactors has expanded to include devices that induce mechanical stimulation, which improves compositional and mechanical properties.

The following sections will profile several published bioreactor designs and experiments, which will help in identifying design intent, areas for improvement, and potential design opportunities.

2.2.1. Current bioreactor designs

1. Seliktar's cyclic strain bioreactor

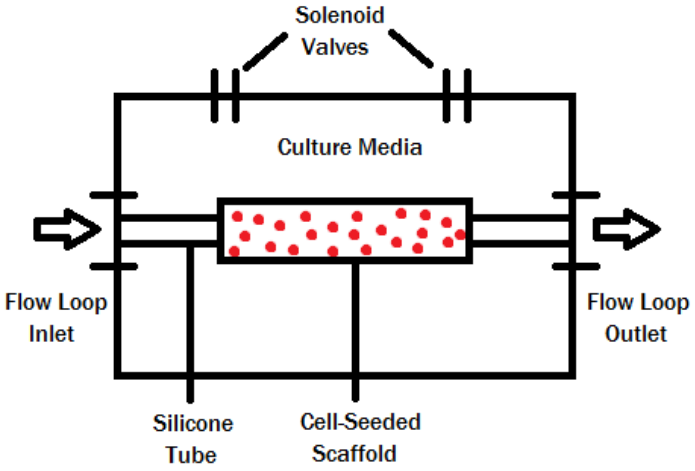


Figure 2. The Seliktar team's final bioreactor design houses the tissue construct on a silicon tube as an external pneumatic control device imparts cyclic strain. Two filters installed into the device allow for gas exchange. (adapted from Seliktar et. al., 2000.)

Seliktar recognized the importance of mechanically conditioning TEBV constructs in order to improve their ECM production and mechanical properties. His team cultured adult rat aortic SMCs embedded in a collagen gel scaffold; once they were mechanically stable, the tissue constructs were transferred into a sterile bioreactor that interfaced with a controlled pneumatic flow loop to impart cyclic strain on the tissue samples.

Seliktar's design (**Figure 2**) consisted of a bioreactor chamber that contained an inlet and outlet port to allow for the mechanical flow. The team's TEBVs were installed on a flexible silicone sleeve and fastened to the ports using sterile sutures. Gas exchange was made possible by two 0.2 µm filters that were installed into the top wall of the chamber. An external compressed air system connected to the bioreactor's inlet and outlet ports using solenoid valves, and provided 5% and 10% cyclic strain to the inner silicon tubing. (Seliktar et. al., 2000)

Seliktar's design yielded TEBVs that exhibited improved cellular alignment and high collagen expression, but experienced large decreases in volume, length, and wall thickness. Still, despite the changes in size, the realignment of cells and collagen in response to mechanical conditioning yielded great increases in

mechanical integrity. Samples conditioned for only four days exhibited an 87% higher yield stress and eight day samples showed a 200% higher yield stress than static samples. (Seliktar et. al., 2000)

2. Kelm improves production time using microtissues

In 2009, Kelm sought to build a bioreactor system that would yield healthy, scaffoldless, small-diameter TEBVs while reducing the production time of previous designs. The team used myofibroblasts and endothelial cells to construct microtissues as an initial platform for the tissue culture. Kelm suggests that microtissues produce ECM quicker and more efficiently. This natural tendency to produce ECM, combined with pulsatile mechanical conditioning of the tissue, would reduce the required maturation time of the cells, and therefore expedite production of mature tissues. (Kelm et. al., 2010)

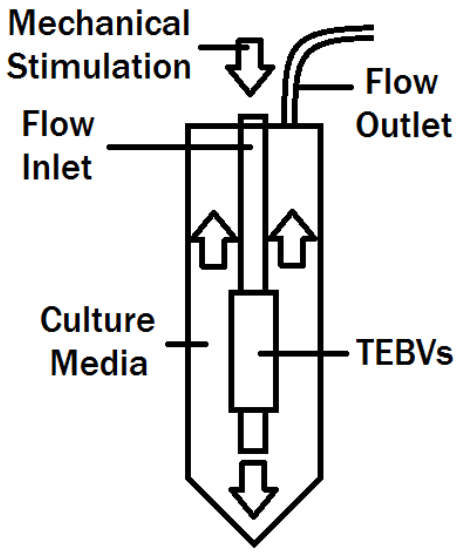


Figure 3. A cross section of the tissue mount, the Falcon tube housing unit, and the path of media flow (adapted from Kelm et. al., 2010)

Kelm's bioreactor design consists of three main components: a pulsatile pump, a medium reservoir, and a structural unit. The pulsatile pump used is interfaced with control unit powered with a 1 bar inlet pressure; it uses cell media as a flow medium. The two stated objectives of the novel assembly device are to grow the microtissues into a three-dimensional tubular shape and to enable circumferential mechanical stimulation. This assembly device is housed in a 50 mL falcon tube with a custom-made stainless steel cap. The assembly component is interfaced with the rest of the system through the steel

cap using silicon tubing (**Figure 3**). Finally, a nutrient medium reservoir is used as a junction between the assembly device and the pump to allow recirculation of the flow medium.

Kelm's design demonstrates that self-assembling microtissue constructs in conjunction with a pulsatile flow loop accelerates ECM production and tissue maturation, as the tissues showed very high collagen expression after only 14 days. This novel concept brings the industry one step closer to the quick and efficient production of autologous TEBV constructs. (Kelm et. al., 2010)

3. Syedain enhances ECM composition

Syedain's team has developed a bioreactor that imparts cyclic mechanical strain on tissue engineered heart valves in an attempt to optimize compositional properties. Syedain's design consists of a latex tube which houses the tissue engineered heart valves. The valves receive mechanical stimulation through a cell media flow via a syringe pump that has been custom-made for this system. A needle valve assists in redirecting the fluid back through the syringe. Media is also supplied to the latex chamber through a perfusion loop that is driven by a MasterFlex peristaltic pump. Using a second loop for media exchange allowed the mechanical frequencies and strains to be adjusted without completely altering the nutrition and oxygen supply to the tissue. (Syedain et. al., 2009)

Syedain's bioreactor yielded tissues that exhibited homogeneous cellularity, which proves that all of the cells were well-nourished and that the dual-flow loop system was successful. Furthermore, the conditioned heart valves expressed 86% more collagen than static control samples, but still fell short by 37% in comparison to native heart valves. (Syedain et. al., 2009)

4. Current device used in the Rolle lab

The current technology used in the Rolle lab at WPI consists of a polystyrene BD Falcon conical tube (**Figure 4 - A**) as a tissue chamber and housing unit for a flexible silicone tube on which the vascular constructs are installed. The cap of the tube has been modified in order to contain a threaded barb, which acts as a platform for the junction between the compressed air chamber (which provides the mechanical loading) and the bioreactor cartridge. The cap system is also fixed with a heat sleeve and anti-leak o-rings in order to ensure the security of the cartridge. Furthermore, an air pressure fitting has been installed into the cap. The final piece of the cap system is a sterile air filter that allows for gas exchange as the tissues are being cultured and conditioned. The bottom of the silicone tubing contains a

needle end cap, onto which a small weight is secured. This grounds the tubing and, consequently, the TEBVs, and helps control the mechanical distension of the system. (Ali et. al., 2009)

One unique feature of the air pressurization design in comparison with the other profiled current

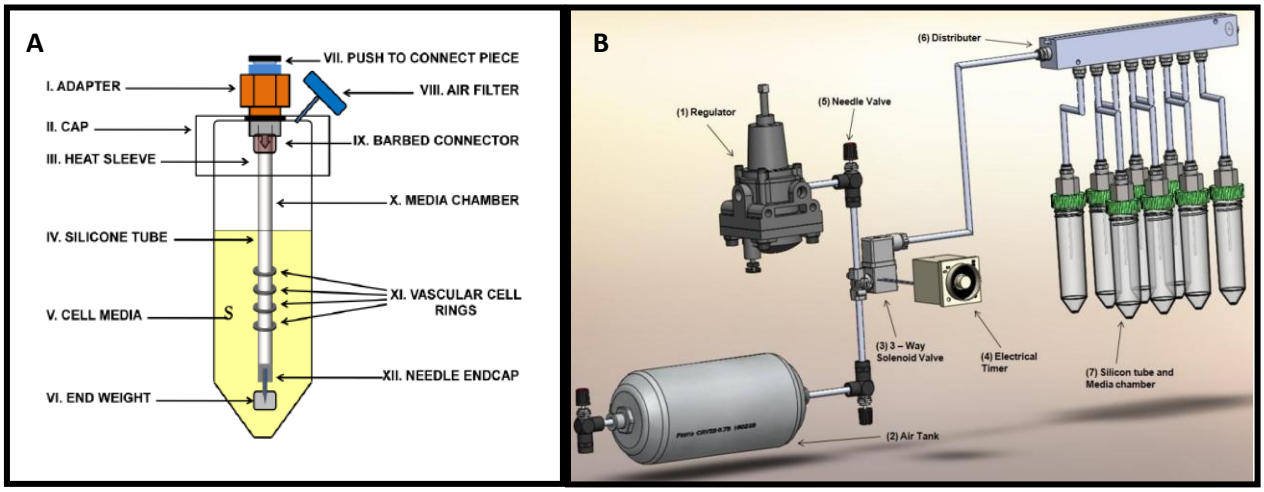


Figure 4. The Ali design uses tube as a tissue chamber, and allows up to eight isolated bioreactor systems to operate at one time. (Ali et. al., 2009)

technologies is the high throughput that it allows. (**Figure 4 – B**) The air pressurization design uses a manifold to distribute the bursts of compressed air among up to eight bioreactor cartridges. This design allows for several isolated tissue cultures to be active and conditioned simultaneously. (Ali et. al., 2009)

2.2.2. Areas for improvement and design opportunities

Several different bioreactor designs, both in the general industry and in the microcosm of the Rolle lab, have exhibited the potential of enhancing ECM production and structural integrity of TEBVs through mechanical conditioning. It is imperative to address the shortcomings of each design in order to optimize the biological performance and economical and industrial efficiencies of these devices.

These issues were partially addressed by Kelm (2010), and they were able to reduce the total production time for a matured vessel, the design still retained a very low throughput for the amount of work that was required to yield a desirable tissue product. The only profiled bioreactor that exhibits the capability to yield a high throughput is the air pressurization design that is currently being used in the Rolle lab at WPI. Even so, the current device has sacrificed sterility (threads warp in the autoclave). It is not designed for efficient and constant media exchange, and does not have the customizable features that are

needed for future studies (interchangeable with a growth chamber; ability to use different tube diameters, etc.).

The MQP team seeks to alleviate these complications by developing and prototyping a design that allows for high throughput of isolated tissue cultures while maintaining the chemical, biological, and mechanical conditions that optimize cell growth and alignment, and ECM production comparable to native tissue.

Chapter 3 – Project Strategy

Our initial client statement challenged us to design a device that overcomes limitations associated with current bioreactors that mechanically condition tissue engineered vascular constructs. It was then our responsibility to conduct an in-depth literature review in order to gain a full understanding of the current technology. As we developed an understanding of the problem, we began compiling a list of objectives and constraints. Utilizing a collection of client interview data, we then applied these objectives and constraints and created a list of functions and means for our device, outlined in Chapter 4. These means were then analyzed and several design alternatives were created. The designs were further scrutinized and with continued research we decided on and refined a final design, which we evaluated through a series of quantitative and qualitative bench top tests.

3.1 Initial Client Statement

The initial statement provided by our project advisor, Marsha Rolle, was: "Bioreactors have been shown to improve the structure and function of engineered tissues by providing conditions that simulate the *in vivo* environment in which the tissue normally exists. In addition, bioreactors can facilitate seeding, organization and culture of cells to support tissue growth and maturation. For tissue engineered blood vessels, bioreactors that provide cyclic, circumferential mechanical loading have been shown to increase cellular alignment and increase extracellular matrix (ECM) synthesis, leading to improved tissue mechanical strength. The goal of this project is to create a cartridge to house a tissue engineered blood vessel that interfaces with a pressurization system that imparts cyclic circumferential strain on the tissue during culture. The cartridge should include an external medium flow loop to provide continuous nutrient supply to the tissue. Ideally, the cartridge should be inexpensive and easy to manufacture, such that multiple cartridges can be used in a single experiment to culture batches of tissue engineered blood vessels. Finally, the cartridge should be interchangeable with the luminal flow cartridge under development by another MQP team and members of the Rolle Laboratory."

3.2 Objectives

Based upon our initial research of past MQPs and published literature, we developed the following objectives:

- *Accurate*: Must produce a strain magnitude within 10% ± 1%
- *High Throughput*: The ability to "condition many rings or tubes simultaneously, run multiple media conditions simultaneously, and/or vary culture duration." (Ali et al., 2009)
- *Adjustable*: Able to condition TEBVs of different diameters and lengths at different frequency and strain levels.
- *Easily Secured*: TEBVs should be easily secured prior to testing.
- *Easily Removed*: TEBVs should be easily removed after testing.
- *Inexpensive*: Both to build (using cheap materials/parts) and to maintain (using less media, durable and reusable materials, energy efficient, less time expense).
- *Easy to Use*: With minimal manual input, using fewer, simpler steps.

With the completion of the literature review, the team then developed a Pairwise Comparison Chart (PCC) which helped us determine which objectives needed to take priority when we reached the conceptual design stage (**Chapter 4**). In total, nine objectives were analyzed in order to establish a set of design priorities. Additionally, the PCCs were issued to our advisor and a graduate student, who as actual clients of the device could further validate the design priorities. Our PCC can be found in **Appendix A – Pairwise Comparison Chart (PCC)**.

As seen in **Table 2**, three areas were determined to be a priority for both clients and the team: accuracy, media supply and waste removal. Accuracy was defined as our device's ability to induce 10 ± 1% cyclic strain. As found in literature, strains of this magnitude significantly increased the mechanical and structural integrity of TEBV samples. (Seliktar et. al., 2000) Both media supply and waste removal were related to our device's ability to sustain cell constructs. It was considered a necessity to maintain an ample supply of nutrients for the cell samples during conditioning. We all agreed that our design must provide constant nutrient supply and allow for repeated replenishment of nutrients. Additionally, all users felt that waste removal was equally important as to maintain cellular homeostasis. These areas of interest are highlighted yellow in **Table 2**. Highlighted red in **Table 2** is an additional area of interest and refers to the amount of effort required to secure tissue samples to our device. As a team we did not feel this was as important as some of the other objectives, however after receiving the client completed PCC, we realized that it may be more important than initially thought. Newly cultured tissue samples are very fragile and during the process of transferring the samples from their culture molds to our device they

can easily break. Both clients decided that our device should facilitate this process. This list of priorities and weighted objectives functioned as the backbone for the development of design alternatives.

Table 2. Client and Team PCC Comparison

Objective	Rolle	Gwyther	Team	Average
Media Supply	8	7	7	7.333333
Waste Removal	7	5	6	6
Accurate	4	8	6	6
Easily Secured	6	5	3	4.666667
High Throughput	2.5	4	4	3.5
Easily Removed	5	2	3	3.333333
Programmable	0.5	1	5	2.166667
Adjustable	0.5	4	1	1.833333
Inexpensive	2.5	0	0	0.833333

3.3 Constraints

In addition to the development of objectives, we also needed to determine our design constraints. Our two most limiting constraints were time and budget. The final deadline for project completion was April 21st, 2011 on Project Presentation Day. Our budget consisted of $368.00, which would have to cover all of our purchased materials for prototype and final design manufacturing. Safety was another priority for all of our alternative designs. The design could not harm the user or cells in any way. The bioreactor must be biocompatible, non-toxic to the cells within it, and contain no possible outlets for injury.

The materials we use in our design must be sterilizable in order to avoid contaminating tissue samples. In addition to sterility, cell viability must be maintained by means of constant media supply to the TEBVs during mechanical conditioning. Waste regulation must also be accounted for in order to guarantee optimal cellular conditions.

After discussion with our client, we determined that the bioreactor must be transparent to allow for imaging and observation during TEBV testing. Transparency aids in the detection of experimental failure, such as: ring failure, culture contamination, or component failure.

Since our device doesn't have a means to self-regulate physiological conditions, such as temperature and humidity, the device must fit within an incubator. Each shelf in the incubator in our laboratory is 40 cm wide x 15 cm high x 40 cm deep. This constraint impacted our designs by limiting the available space that our device can occupy.

3.4 Revised Client Statement

After an improved understanding of the assigned problem, and an extensive knowledge of the current technology, we were able to apply our metrics and overall project strategy, and improve upon our initial client statement. The resulting client statement reads as follows:

> To design a bioreactor used in conjunction with a pressurization system that distends the inner diameter (2mm) of tissue engineered blood vessel rings and/or tubes (1cm long) by 10% (± 1%) at a frequency of 1 Hz. This will be accomplished by means of cyclic circumferential strain, which increases cellular alignment and ECM synthesis. Multiple cartridges should allow for media regulation in a safe, sterile, leak-proof *in vitro* setting that accurately simulates an *in vivo* environment. Finally, the device should be inexpensive and easy to manufacture and use.

With this enhanced definition of the problem we then entered the development stage which is completely outlined in the next chapter.

Chapter 4 – Alternative Designs

Based on our initial client statement, we developed constraints, objectives, functions, and means by which to formulate design alternatives. Using the air pressurization system designed by Ali, 2009 (**Figure 5**) as a template and the designs of other successful bioreactors as models, the team evaluated a number of design alternatives.

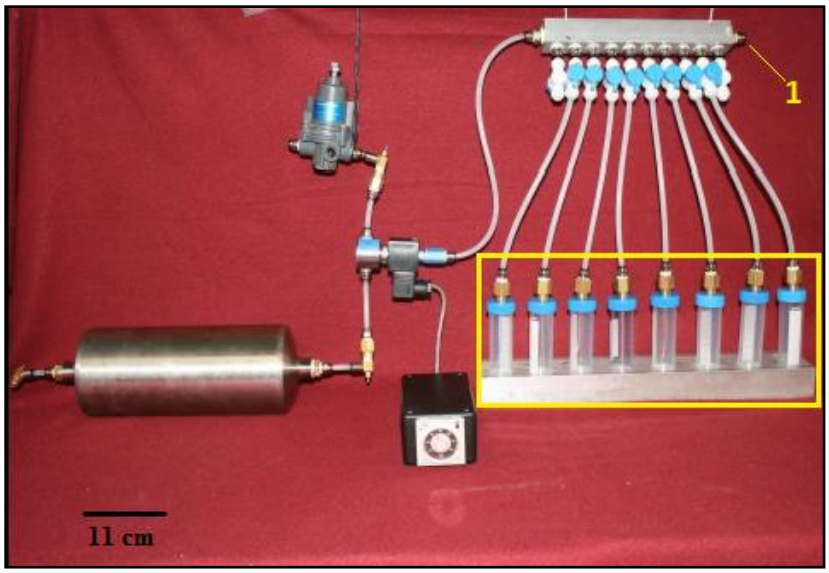


Figure 5. Air pressurization system (Ali et. al., 2009)

After studying the Ali tissue chamber design outlined in yellow in **Figure 5**, we determined that the design had several faults. The silicone tube fixed to the threaded cap was dangling within the BD Falcon conical tube (**Figure 4 - A**). Without any effective means of fixing the free end of the silicone tube, there was nothing to prevent it from curling or knocking against the sides of the conical tube. The only means of holding the silicone tube taut was via an end weight attached to the needle endcap which was glued into the free end of the silicone tube. This endcap was still not effective at preventing the free swinging of the silicone tube during transport. The vertical orientation of the tissue chamber makes the tissue rings susceptible to gravitational forces that can merge multiple rings together or possibly even slide them off of the silicone tube entirely. A horizontal orientation would eliminate this problem.

Other drawbacks of the pressurization system include its size and user-friendliness. The pressurization system makes use of compressed air, which is stored in large tanks in the lab. This takes up lots of space in the lab and is loud and disruptive in the user's workspace. Furthermore, a heavy aluminum base

makes the device awkward to transport. Finally, the device was designed so large that it cannot fit in a laboratory incubator and therefore cannot be used for culture experiments. Given these limitations of the initial design, we chose to pursue a completely novel approach to our project.

During design conception, we made an effort to use common parts (standard sized screws, syringes, tubes, motors, etc.) to reduce costs of manufacturing and materials, expedite the design process, and to minimize production times.

4.1 Functions (Specifications)

With a firm understanding of our objectives and constraints we then began defining potential functions and means which could be applied to design alternatives. We decided to separate the design functions into two categories: functions/means for the tissue chamber and functions/means for the mechanical conditioning system. Below is an itemized list of our proposed design functions.

Tissue Chamber Functions

- Provide nutrients to cells
- Remove cell waste
- Allow access to and removal of tissue rings/tubes
- Allow gas exchange
- Compatible with a pump

Mechanical Stimulation System Functions

- Cyclically distend tissue samples by 10%
- Run during the duration of mechanical conditioning of samples
- Consistent (must strain at 10 ± 1% per cycle)
- Adjustable pressure controls (per tissue chamber)
- Compatible with tissue chamber

In order to visualize these functions, after making a list, we then created a Morphological Chart (**Table 3**) and continued to expand upon the potential means for each function.

Table 3. Morphological Chart

Functions	Mean 1	Mean 2	Mean 3	Mean 4
Tissue Chamber				
Media Provision	Submerge in Media	Rotisserie Media Pool	Porous Mandrel	
Cell Waste Removal	Aspirator	Drainage Flow Loop	Filtered Reservoir	
Tissue Sample Access	Removable Lid	Individual Tissue Chambers	Open Container	
Gas Exchange	HEPA Filter	Expose to Air	Petri lid	
Humidity Regulation	Exposure to Incubator			
Pump Compatibility	Syringe Pump	Peristaltic Pump	Mechanical (CAM/Motor) Pump	Air Pressurization System
Mechanical Conditioning System				
Cyclic/Circumferential Loading	Syringe Pump	Peristaltic Pump	Mechanical (CAM/Motor) Pump	Air Pressurization System
Consistent Work Output	Programmable	Leak Proof	Mechanically Adjustable	Spring
Adjustable Pressure	Programmable	TRIM Pot	Bread Board w/ Variable Resistors	Motor w/ Turn Off Switches
Tissue Chamber Compatibility	Fluid Two-Way Valve	Quick Connect Tubing Couplings		

4.2 Conceptual Designs

In order to meet our outlined objectives, we broke up our bioreactor system into two major components: a mechanical conditioning system and a tissue culture device. Each had their own set of objectives and functional requirements.

4.2.1. Mechanical conditioning system

The purpose of the mechanical conditioning system is to use a flow medium to distend an elastic tube on which engineered tissue samples are mounted. Distensible tubing has been used in past research as a means of imparting mechanical strain on tissue engineered constructs (Niklason et. al., 1999). The elastic tube is generally seeded with tissue constructs and inflated by air or liquid to distend the tube, and thus the tissues mounted on the tube.

1. Peristaltic pump

Using the Syedain heart valve bioreactor design (Syedain et. al., 2009) as a model, the team considered the use of a peristaltic pump as a means to provide circumferential mechanical stimulation to the TEBVs. The pump operates by using a rotating cam (**Figure 6 - 1**) to flex plastic tubing (**2**) that is fixed to a frame (**3**). The design would incorporate inlet and outlet channels (**4**, **5**) in order to allow for a continuous circuit of flow medium.

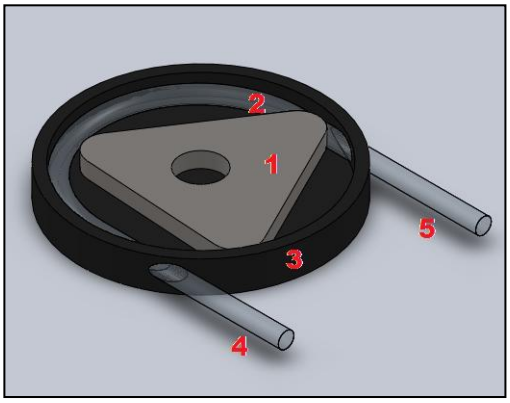


Figure 6. Peristaltic pump

Using a peristaltic pump as a design component is very appealing, as it is an existing technology and is programmable. However, one major drawback is that existing models do not offer high throughput capabilities.

2. OctoPump

The next design alternative was conceived by reverse-engineering a syringe pump. The design consists of eight syringes (**Figure 7 - 1**) housed in a circular frame (**2**). The pump uses an offset rotating cam (**3**) located in the center of the frame to depress the plungers of the syringes. The displacement of a medium would inflate a plastic tube on which the tissue rings are housed, thereby providing the desired distension.

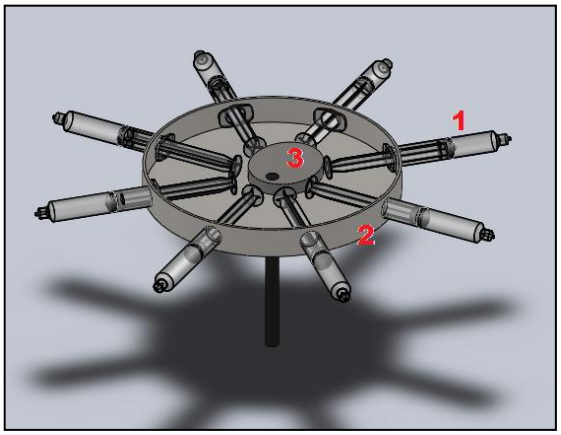


Figure 7. OctoPump

This novel design allows for high throughput of isolated samples, and the cam device could be manipulated to adjust flow frequencies and volumes. However, in order to remove a single sample the user would have to deactivate the entire system.

3. Wheel and arm motor

Another design that mimics a syringe pump is the wheel and arm motor assembly. A small mechanical motor (**Figure 8 - 1**) spins a machine wheel (**2**) attached to a flat arm (**3**). The flat arm's far end is attached to a housing device that is fixed to several syringe heads (**4**). As the wheel spins, there is a horizontal translation in the syringe heads, causing them to depress and retract. The flat arm can be fixed to the wheel at any of several points (**5**), resulting in different radii and therefore different flow volumes.

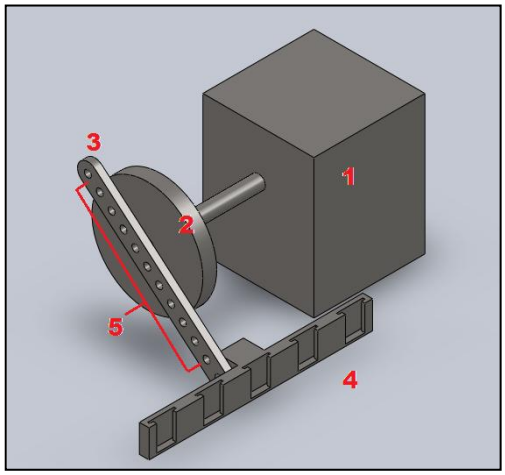


Figure 8. Wheel and arm motor

This design concept is appealing as it combines several simple existing technologies for a novel application. It also serves as an effective, high-throughput alternative to the syringe pump, which was deemed out of the team's price range. Some predicted complications include size and isolating single syringes or assembly systems.

4. Solenoid flexion device

The next preliminary design was a solenoid flexion device. The design consists of a battery-operated solenoid (**Figure 9 - 1**) suspended above the plastic tubing (**2**) which contains the tissue samples. Fixed to the solenoid is a rubber press (**3**). The size of press would be chosen depending on the volume of water in the tubing that needed to be displaced in order to inflate the tubing and achieve the desired 10% distension of the tubing and TEBVs.

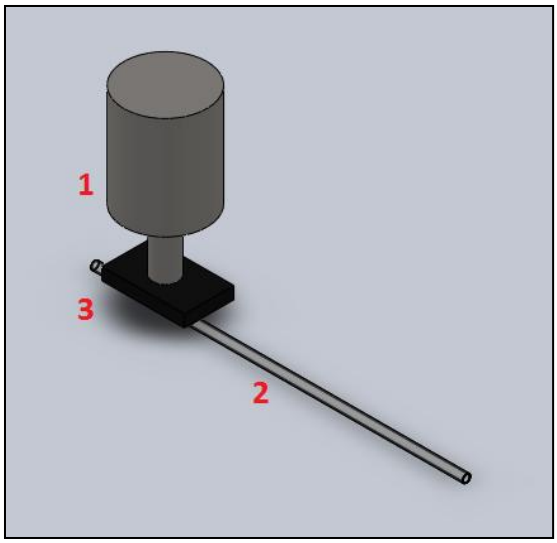


Figure 9. Solenoid flexion device

While this design assembly eliminates several parts and fixture points – and in turn, eliminates opportunities for the device to fail – it was deemed unfit for this project. It was discovered that it is very difficult to program a solenoid to function at speeds as low as 60 RPM, which is required for the mechanical conditioning to

take place at 1 Hz. Furthermore, the movements of the solenoid are abrupt, irregular, and may be abrasive to the silicone tubing. (J. O'Rourke, personal communication, October 2010)

5. Motor and cam system

The team's final conceptual design for the mechanical conditioning system involves a small motor (**Figure 10 - 1**) that contains a cam and cam collar (**2**). As the cam spins, it remains tangent with the head of a syringe plunger (**3**) that displaces a flow medium from a 1 mL syringe (**4**) through a series of luer fittings (**5**) and into a flexible plastic tube (**6**) that houses the tissue rings. The tube is secured at the distal end using a pinch clamp (**7**). The plunger is fitted with a compression spring (**8**) that makes it to retract, stay in contact with the cam, and allows for cyclic pumping of the syringe.

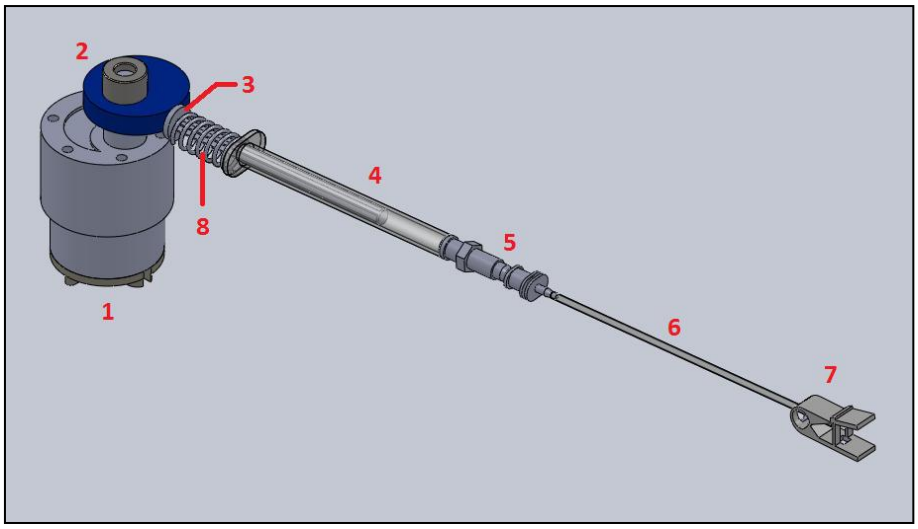


Figure 10. Motor and cam system

One highlight of this design is that the user may choose different cams or motors to customize the parameters of the mechanical conditioning. For example, a larger cam would displace a larger volume of medium through the syringe, thereby causing a greater distension of the tissue rings. A motor may be run at lower or higher voltages to increase or decrease the frequency of the conditioning.

4.2.2. Tissue chamber

All of the designs for the mechanical conditioning system involve the tissue samples being mounted on a flexible plastic tube that is inflated in order to achieve the desired distension. The final assembly therefore requires a unit made from biocompatible materials that safely houses the tubing and cells, prevents contamination, allows gas exchange between the cells and the environment, and interfaces effectively with the mechanical conditioning system.

The current design used in the Rolle lab in conjunction with the air pressurization system contains a number of flaws. The design fails to secure the silicone tubing at both ends. Fixing the silicone tube at both ends and keeping it taut prevents the tube from moving and ensures that the displacement of water through the tube leads to inflation and distension. Additionally, the current design offers no outlets or means for waste removal and media exchange.

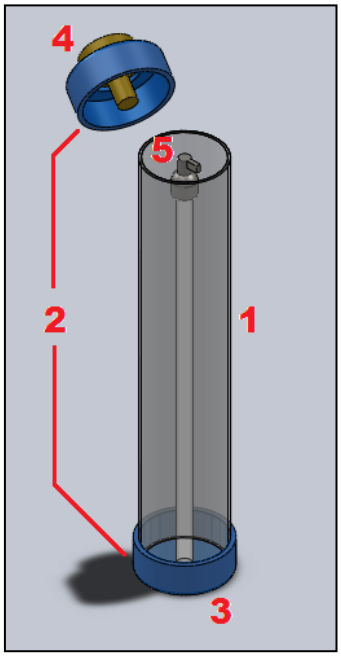


Figure 11. Double threaded cartridge

1. Double threaded cartridge

The first design alternative was based on the tissue chamber from the air pressurization design, which uses a series of polystyrene Falcon conical tubes. Because these units are small and create isolated environments for each tube and set of cells, there are many possibilities for a high throughput assembly. The design consists of a hollow tube (**Figure 11 - 1**) with threaded caps on each end (**2**). One cap would permanently house the distal end the silicone tube (**3**). The second cap would function much like the current design, as it would be interfaced with a fitting that leads to the pump system (**4**). A lock-and-key pin mechanism (**5**) fixes the proximal end of the silicone tube to this cap. Because the tubing is fixed to the threaded caps before they are both screwed onto the Falcon tube, the tubing is subjected to torsion and possible tearing or deformation. Therefore, the biggest challenge associated with this design would be to not compromise the safety of the silicone tube and the tissue sample when fastening the threads.

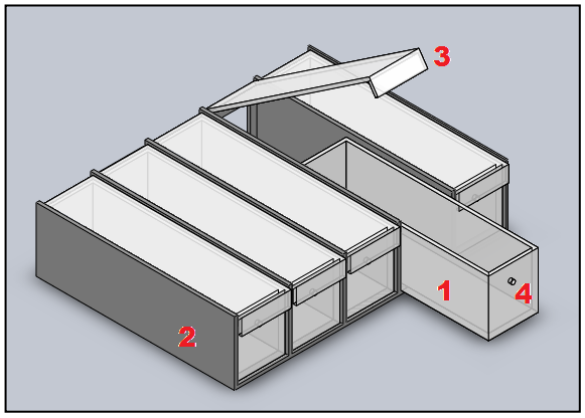


Figure 12. Removable compartment assembly

2. Removable compartments

This design uses an original assembly of isolated, removable plastic compartments (**Figure 12 - 1**) that fit into a containment unit (**2**). The frame separates the compartments, acts as a junction between the pump system and cartridges, and provides hinged covers for each compartment (**3**). The hinged covers

loosely sit atop the compartments, which allows for gas exchange and provides easy access to the interior for aspirating and changing out cell media. Finally, the fronts of the compartments will contain a small opening (**4**) above the media pool to which the silicon tubing will be fixed by a clamp located outside the tissue chamber.

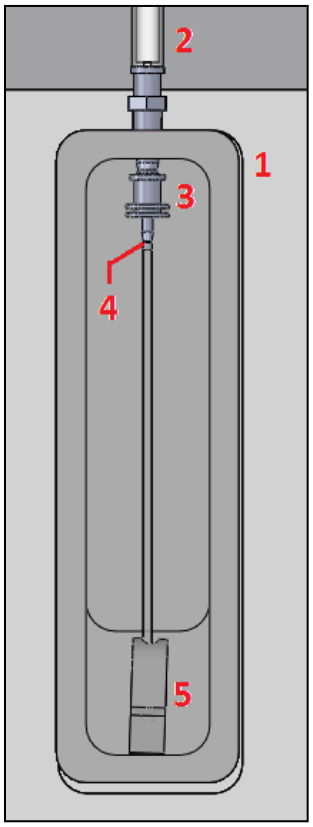


Figure 13. Milled tissue chamber

3. Milled tissue chambers

This conceptual design consists of milled plastic tissue chambers (**Figure 13 - 1**) which interface with a mechanical conditioning system's syringe (**2**) through a series of luer fittings (**3**). The flexible plastic tubing is attached to the luer fittings using heat shrink-wrap tubing (**4**). The distal end of the silicone tubing is clamped using a pinch clamp (**5**) that is fixed to the bottom of the chamber using a stainless steel dowel.

4.2.3. Design assembly

1. Wheel and arm assembly

The design is driven by the wheel and arm motor (**Figure 14 - 1**) as described in **Section 4.2.1.-3**. The flat arm is interfaced with a fixture that holds the heads of several BD 10cc syringe plunger heads (**2**). Displaced flow medium is transferred from the syringes into the tissue-mounted silicone tubing located in the compartments of the removable compartment assembly (**3**), which will be constructed as described in **Section 4.2.2.-2**. The combination of these design alternatives allows for high throughput, isolated systems for the simultaneous operation of independent experiments, and high customizability.

One problem that arises with this design is that a single compartment cannot be removed without shutting off the pump for the other compartments. Also, even if a single compartment can be removed, there is nothing stopping the water in the silicone tubing from spilling out. The team conceptualized a system that may overcome these design barriers. Each syringe would pass the flow through a three-way cross flow valve (**4**). The top end of the valve will lead into a BD Falcon polypropylene tube, which would be fixed with a loaded silicone tube (**5**). When a tissue chamber needs to be removed, the flow can be redirected using the cross flow valve into the Falcon tube, which serves as a temporary reservoir for the

flow medium. When the tissue chamber is put back into the system, the flow can be directed again back into the chamber. When the user wants to remove a single compartment, he or she will simply redirect the flow to the reservoir, stopping the flow into that cartridge without interrupting the others. This still leaves the problem of water leaking from the system into the incubator following removal of the compartment. The remaining end of the three-way valve will be fitted with a quick-coupling fixture that only allows one-way flow.

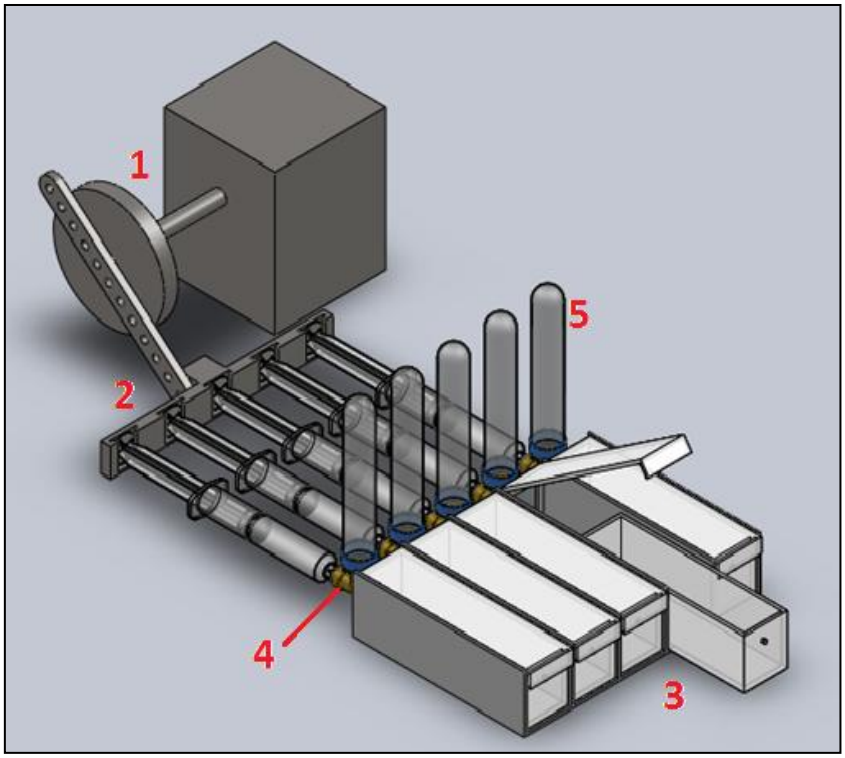


Figure 14. Wheel and arm assembly

2. Pyramid assembly

The team conceptualized modifications to the wheel and arm design. In order to increase user control over each TEBV sample, the team sought to create isolated systems for each tissue chamber. In order to achieve this, the team concluded to use small gear-box hobby motors (**Figure 15 - 1**).

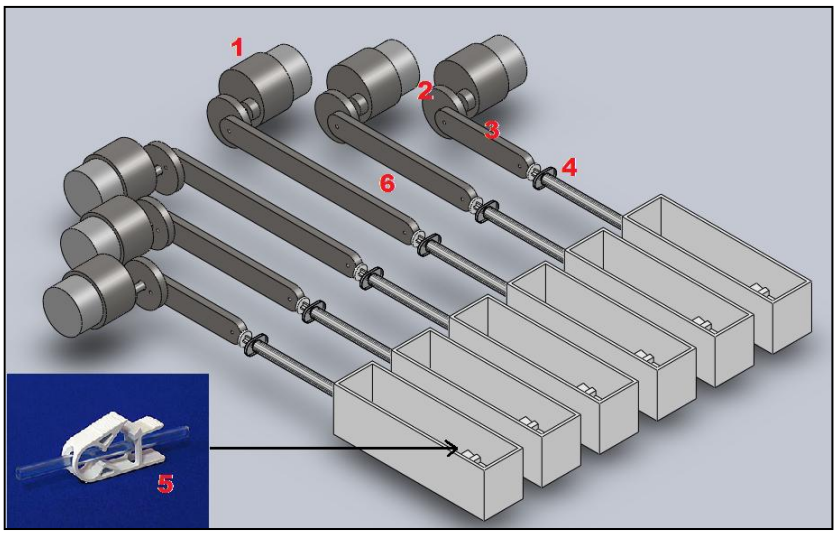


Figure 15. Motorized pyramid assembly and pinch clamp (ZManCorp.com)

These motors can be purchased off the shelf and run at 60 RPM. Again, a machine wheel (**2**) and flat arm (**3**) would be fixed to each motor. The opposite end of the flat arm would attach to the plunger of a 1-mL syringe (**4**). As the motors rotate the wheel, the flat arms would undergo a horizontal translation, causing the plungers to depress and retract. These syringes interface with milled tissue chambers (see **Figure 13**) which contain an internalized pinch clamp (**5**). The motors are arranged in a staggered 'pyramid' formation in order to minimize the amount of occupied space while maintaining the linearity of the system and allowing the user to easily access the tissue chambers. To accomplish this, three different sized flat arms are required (**6**).

In order to improve manufacturability, the team dismissed the flat arm component of the design en route to developing the following design alternative:

2. Motor-cam series

The motor-cam series builds on the concept of the pyramid assembly, but simplifies it by reducing the number of parts and fittings, improving manufacturability, and internalizing the mechanism in order to make a compact, customizable, user-friendly device. The assembly consists of a plastic base (**Figure 16 - 1**) which houses six 60 RPM gear-box motors (**2**). Each motor is fixed with a cam (**3**) which rotates and cyclically depresses the plunger of a 1-mL syringe (**4**). Again, the design employs tissue chambers and pinch clamps as seen in **Figure 13**. Six of these tissue chambers (**5**) are housed in a removable UHMWPE tray (**6**) that fits into the base.

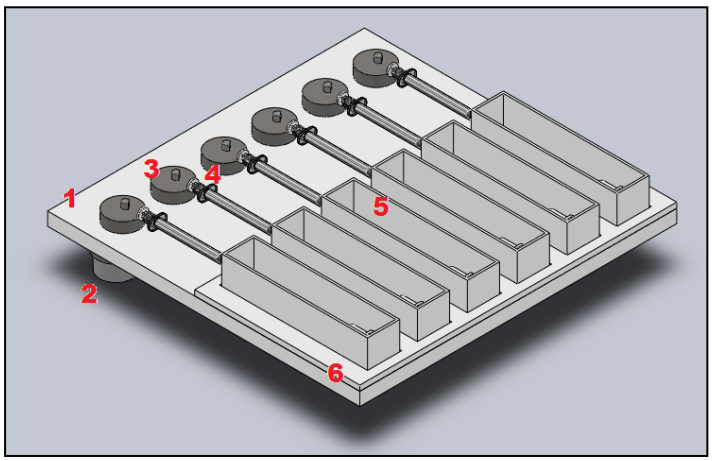


Figure 16 Motor-cam series

4.3 Comparison of Design Components

In addition to comparing alternative designs as a whole, we compared individual components used within these designs to aid in our selection of pieces for a final working assembly. We compared these components using research, interviews with experts and clients, experimental testing, and general knowledge obtained during the course of our project.

1. Distensible tubing

We were given fifty feet of clean, not sterile, 1.4732 mm inner diameter x 1.9558 mm outer diameter silicone tubing from Specialty Manufacturing Inc. (SMI) at the start of our project. We decided to build our design around this source of distensible tubing due to the Rolle Lab's experience handling this particular size and brand.

Silicone tubing has been shown to be an effective means of distending tissue engineered constructs (Niklason et. al., 1999). The inner diameter of the tissue rings being used in our project (2 mm) match almost precisely with the outer diameter of the silicone tubing (1.9558 mm), leaving an extra 0.04 mm to aid during tissue ring loading. Loading involves delicately placing a tissue ring or tube around the outer circumference of a sterile distensible tube. The tube is then inflated or pressurized continuously at 10 ± 1% of its outer diameter at a frequency of 1 Hz over a 3-7 day period (in our studies). In the event that the tissue rings adhere to the surface of the silicone tubing during distension, they can be detached simply by stretching on either end of the silicone tube (M. Rolle, personal communication, September, 2010).

Silicone tubing is autoclavable, inexpensive (at ~2 cents per 10 cm length), disposable, can interface with syringe tips, and will not leach when soaked in cellular media for long periods of time.

2. Mechanical conditioning device

We considered three different device designs before reaching a final decision for a means of distending the silicone tubing. First, an air pressurization system designed by the Ali MQP in 2009 was considered as a means of distending silicone tubing via air flow. This device used a pressure control system which provided a constant high pressure of 26 psi which obtained the 10% strain on the silicone tubing. A three-way solenoid valve controlled by an electrical timer switched back and forth between the system's high (26 psi) and low (4 psi) pressure inputs at a frequency of 1 Hz. The use of air pressurization proved to have its drawbacks with the Ali MQP's system. The entire system was large and complicated, involving numerous connections between the air compressor, pressure control devices, distribution manifold, and tissue chambers. The pressurization system shown in **Figure 5** consists of every part of the system except for the tissue chambers outlined in yellow. The overall size of this system makes it incredibly difficult to run within an incubator, as well as incredibly noisy. While there is an option for running tubing outside of the incubator, the size of the distributor (labeled as **1** in **Figure 5**) and bulk of

the remainder of the system would make it difficult to arrange within and around an incubator. The greater the number of connections between the system and the tissue chambers, the greater the chance of leaks, loose fittings, and difficulty in monitoring air flow and air levels. (Ali et. al., 2009)

Our second option was to use a solenoid coil actuator to displace a specific volume of water within a silicone tube to achieve 10±1% distension. Solenoids work by creating a magnetic field around a solenoid coil which moves a metal armature positioned in the middle of the circular coil. The armature is forced down by a spring, pushing the stem and base onto a silicone tube filled with water positioned beneath the solenoid. The entire system is pictured in **Figure 17**. The magnetic field can be controlled to push the armature at a rate of 1 Hz based on the number of loops in the solenoid coil. There are several problems associated with this system, namely that solenoids operate in a very abrupt manner (J. O'Rourke, personal communication, October 2010). The movement of the armature and flexion of the spring are not smooth in motion, which could cause damage to the silicone tube and friction in the system. Using a solenoid in a moist, humid environment may disrupt solenoid function as well. The affects of electromagnetic fields on TEBV growth is also unknown and could potentially be problematic.

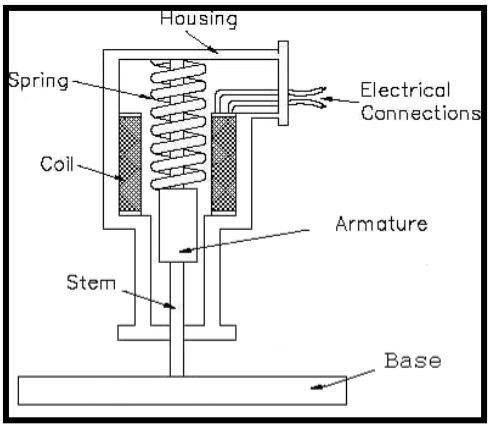


Figure 17. Solenoid actuator (Edited from tpub.com)

Our final option is a motor, pictured in **Figure 18**. Motors can effectively push syringe plungers when attached to a cam and/or flatbar assembly. One motor could be used to move multiple syringes at once, or multiple motors can be used to move syringe plungers individually. The movement of these plungers at 60 RPM results in distension of the silicone tubes at a frequency of 1 Hz. When preloaded with water or media, the silicone tubes can be distended by 10±1% depending on the length of the flatbar or dimensions of the cam.

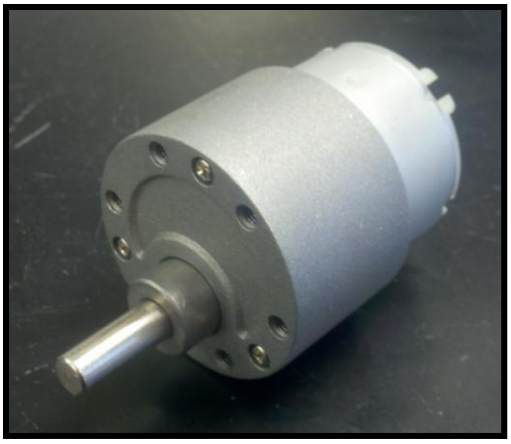


Figure 18. 12V DC 60 RPM high torque gear box electric motor

We chose to use the motor as our method of mechanically distending the silicone tube due to its low cost (see **Appendix F** for pricing), adjustability of frequency, smooth and quiet operation, size (37 mm in diameter, 70 mm in height), and simplicity over the other two design alternatives. Using multiple motors further improves the design by allowing for variation of separate tissue chamber conditions during experimentation. By using multiple motors, individual chambers can be shut off or set to run at a slower speed with the flip of a switch or the turn of a knob. We found during bench-top testing that our motor (**Error! Reference source not found.**) ran very quietly and without complications for 7 days on its own. otors can be positioned in a variety of different ways to achieve the same function, allowing for more design flexibility.

3. Tissue chamber

We narrowed our design options down immediately when deciding between using tissue chambers where the cells would be seeded on a vertical silicone tube (such as in the Ali 2009 MQP) versus being seeded horizontally. We eliminated a vertical tissue chamber design based on our assessment of the air pressurization system used in the Ali (2009) MQP introduced in the beginning of this chapter. Our final concern with the vertical tissue chamber design of the Ali MQP is the amount of wasted cell media used in the 50 mL conical tubes (~35 mL of media used if filled as shown in **Figure 4**). A horizontal design in which enough media would be used to submerge the tissue rings would reduce the costs related to cell media usage.

The only stated concern of the Ali MQP team regarding horizontal tissue chamber arrangements is “the chance that flexible tubes will not remain completely horizontal when inserted into the media” (Ali et. al, 2009). We saw a way to overcome this problem, and so we chose to pursue horizontal tissue chamber arrangements. Our only foreseeable challenge with horizontal chambers was in finding a way to submerge distensible tubing (loaded with tissue rings) within the media in the chambers without leaking. All chamber designs must allow for tissue ring submersion and interface with the motor-cam mechanical conditioning device. We considered two different design variations for a horizontal tissue chamber based on our decisions to eliminate vertical designs and use motors as a mechanical conditioning device.

The first tissue chamber design involved using a solid block with separate chambers milled out of it, and the second involved having separate removable chambers housed on a removable tray (**Figure 19**). Both designs are relatively easy to manufacture through milling and sawing and both have removable components. The tissue chamber with separate, removable chambers was deemed to be a better option for our design. By having separate chambers, the user could have the option of removing a single chamber from the system in the event of sample contamination or a problem with the mechanical components. In the event that a chamber is damaged in some way, it can simply be replaced, eliminating unused or contaminated space in the system. Both tissue chamber designs would interface with the motor-cam mechanical conditioning device through a hole in one of its walls. An air and water-tight seal would allow for the silicone tubing to pass into the tissue chamber for submersion, while maintaining a sterile environment within the tissue chamber itself.

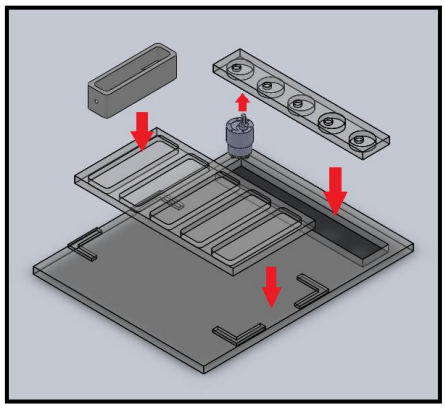


Figure 19. Individual removable chambers on a removable tray

One function of the tissue chambers is to allow for the changing and replacing of media. Both the single unit and individual chambers function similar to culture dishes, in which media must be manually aspirated and added to feed the cells contained within them. The chambers would be complete with loose fitting lids, which act as culture dish lids in permitting gas exchange and offering protection from potential airborne contamination in non-sterile settings. Both the single unit and individual chambers would facilitate media change quite easily with these design considerations. The single unit acts as a tray

in itself while the individual compartments are housed on a removable tray, allowing for all chambers to be moved at once for both designs.

We chose to use the individual, removable chambers on a removable tray to do its greater flexibility for a variety of situations that could occur in the laboratory during testing.

4. Mechanical attachment to motor

We considered two different options for a means of mechanically pushing the syringe plunger using a motor. The first conceptual design used a cam and flat arm design. We would manufacture a cam of appropriate dimensions and attach and clamp it to the shaft of the motor via a D-shaped hole at the center of the cam. In order to achieve 10% distension, the syringe plunger would have to be moved by a certain distance depending on the size of the syringe and the volume of fluid needed to distend the silicone tubing. The distance that the plunger must be moved is equal to the distance between the center of the D-shaped hole and the center of the hole drilled to connect the flat arm to the cam. The other end of the flat arm connects to a syringe plunger, the movement of which controls the distension of a silicone tube and the tissue rings encircling it. Cams and flat arms can be manufactured relatively easily to whatever specifications are desired. Flat arms can be as long as necessary to accommodate for individual motor spacing, although the longer the flat arm, the larger the overall device. The cam and flat arm design can be seen in **Figure 20**.

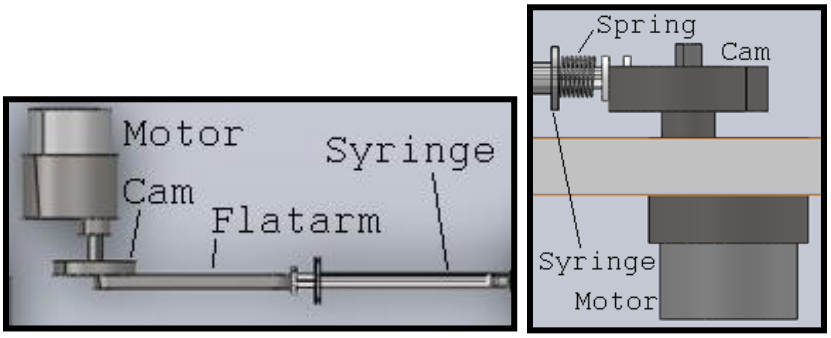


Figure 20. Top view of cam and flat arm design (left) and side view of cam design (right) for motor-syringe attachment

Our second conceptual design was based on using just the cam without a flat arm. The cam dimensions would be calculated in the same way as for the cam and flat arm design. The cam would rotate, depressing the plunger of the syringe with its longer side. A compression spring controls the plunger's

extension as the cam rotates to its shorter side. Springs will be necessary to ensure that the syringe plunger maintains contact with the cam and that the plunger fully extends back to its original position within the one second time frame required for running at a frequency of 1 Hz. The spring must be big enough to fit around the plunger, yet not exceed the size of the plunger head in order to remain positioned correctly between the syringe barrel and plunger during conditioning. By switching out the cam for a different sized one, the volume of water displaced by the syringe can be catered to the user's needs. The elimination of the flat arm in this design allowed for us to shorten our design considerably. In theory, the cam design is more compact, it allows for the motor to be inverted and anchored into the base of the device to further save space. The use of less material for the base of the device saves material costs, and the odds of mechanical failure are reduced by limiting the number of mechanical connections in the overall system.

5. Syringe tips

We considered three different syringe tips as a means of connecting our syringe to the 1.9558 mm inner diameter silicone tubing.

Blunt end metal syringe tips (2 mm diameter; **Figure 21, right**) were first considered due to their similarities in diameter to the silicone tubing we were using. These tips effectively interface with a large variety of syringes, including the 1mL luer-slip and luer-lock.

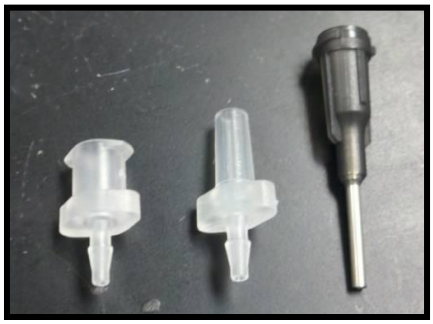


Figure 21. Pictured left to right, 1/16" inner diameter female barbed luer-lock tip, 1/16" inner diameter male barbed bayonet tip, and 2mm blunt end metal syringe tip.

Problems arose with this type of syringe tip when trying to build it into the wall of the tissue chamber. Any tip would have to be sealed into the wall of the tissue chamber to allow for silicone tube submersion and easy connection from the sterile tissue chamber to the syringe and mechanical

conditioning system. Mounting tubing connectors also helps keep the entire system in line, so fluid flows in one direction out of the syringe and the tubing is held taut off the bottom of the tissue chamber to prevent tissue ring damage. In the event of syringe tip or silicone tube failure, there would be no way to remove the 2mm blunt end metal syringe tip from the tissue chamber wall without replacing the entire tissue chamber. Similarly, if the silicone tube were to become worn out due to repeated use, damaged during autoclaving, or otherwise harmed, the 2mm metal syringe tip would have to be removed and replaced.

Figure 22. Syringe-silicone tubing connectors

Our plan to combat these problems was to use a two-tip, male-female luer fittings. The female tip would be secured into the wall of the tissue chamber and fixed there permanently, while the male tip would be permanently attached to the silicone tubing and lock onto the female tip during experimentation. The first male-female luer parts we considered were 1/16" inner diameter barbed luer-lock male and female tips (**Figure 22**) capable of interfacing with luer-slip syringes and silicone tubing. The 1/16" inner diameter luer fittings is approximately 1.6mm, which is as close to 1.4732 mm inner diameter of the silicone tubing as standard parts would allow. The tight fit of the silicone tubing over the luer barb further aided in leak proofing the system. The wider opening of the luer tip leading into the narrower inner diameter of the elastic tubing caused some problems with tube failure when distended past 20% during testing. This gives the user the option to inflate the tube up to 120% of its original diameter in order to increase the strain magnitudes imparted on the tissue rings. Problems arose when we attempted to secure these luers into the wall of the tissue chambers. The luer pictured on the right in **Figure 22** was nearly impossible to permanently mount into the tissue chamber wall.

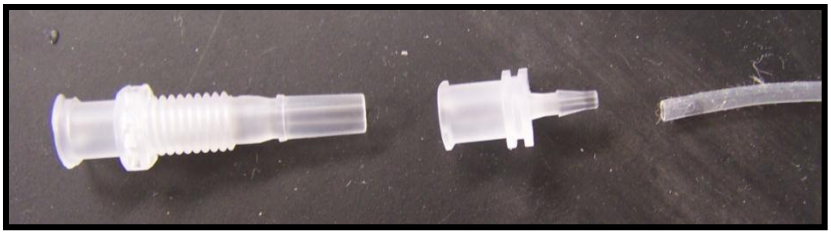


Figure 23. Threaded syringe-silicone tubing connectors.

Due to the problems encountered with the 1/16" inner diameter barbed luers, we considered alternative interlocking luers. We chose to use a ¼-28 threaded luer to fix into the tissue chamber wall that would interface with a 1/16" inner diameter barbed luer-lock tip (**Figure 23**). These luers allowed for permanent fixture into the tissue chamber and easily interfaced with the 1 mL syringes we had been using throughout bench top testing. The 1/16" inner diameter barbed luer-lock tip was heat shrunk to the silicone tubing in the same manner as described in Ali, 2009. The threaded luers were leakproof based on bench top testing over a period of 12 hours. This was accomplished by threading the luers into the tissue chambers, attaching a 1 mL syringe to the outside opening of the threaded luer, and filling the chambers with water.

The fittings are manufactured from autoclavable plastics and come equipped with a barbed tip, which aids in securing the silicone tubing onto the luer fitting and prevents fluid flow beyond the barb. Leakproof testing involving manual fatigue testing of the luers under fluctuating silicone tube pressure for 20 minutes as well as 16 hours of motor-cam distension confirmed the ability of interlocking luer fittings to prevent fluid leakage from the system. By using both male and female luer fittings, we can change out overused or damaged silicone tubes for new ones without having to disassemble or replace any major components of the device. The female 1/16" inner diameter barbed bayonet fittings can be permanently connected to silicone tubing, autoclaved in single-use packets, used for experimentation, and then disposed of. The more easily replaceable standard parts we use in our design, the better the durability and lifespan of our device before standard wear renders it non-functional.

6. Mechanism for attaching silicone tubing to syringe tip

When preloaded with water and clamped at its free end, the silicone tube slides off of the syringe tip due to pressurization from depressing the syringe plunger. To attempt to correct this, we considered three different approaches for sealing the silicone tubing to the syringe tip(s) to prevent leaking and

detachment of the tubing. First, a suture was tied around the silicone tube above the barb on the tip of the 1/16" female luer fitting (**Figure 24**). This method failed. Silicone glue was then applied to the tip shaft of the 1/16" female luer fitting below and above the barb. The silicone tube was then forced onto the luer tip over the barb and set to dry for 48 hours. A second sample using the 2mm blunt end metal syringe tips was also used. Silicone glue failed to prevent leakage at the syringe tip-silicone tube interface (**Figure 24**).

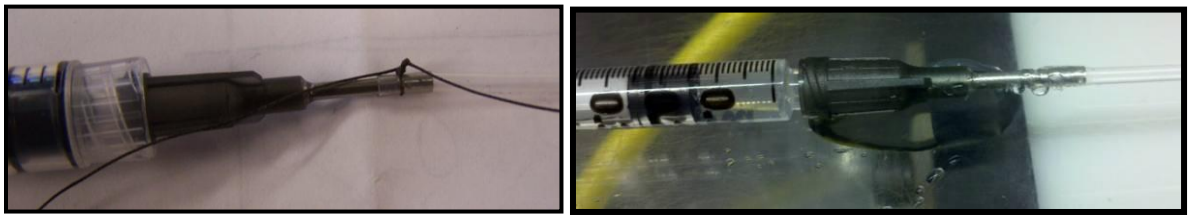


Figure 24. Using sutures (left) and silicone glue (right) to attempt to seal syringe tips to silicone tubing

Ali (2009) found an alternative to these methods. They used biocompatible 1/8" diameter, transparent, heat shrinkable tubing with a 2:1 shrink ratio to seal the silicone tubing to the rest of their pressurization system. After extensive testing involving preloading different syringes, luers, and silicone tubing with water, clamping the distal end of the silicone tubing, and pressurizing the tube past 20% distension (Section 5.1), we determined that heat shrink tubing was our best option and proved to prevent any leaking or detachment of the silicone tubing from the 1/16" female luer fitting.

However, one limitation to this method exists. When manufacturing these syringe tip-silicone tubing parts, one must use a heat gun to adhere the heat shrink tubing to the syringe tip-silicone tubing junction by heating the heat shrink tubing above 175^{o}C (see Appendix A for specific instructions). At such high temperatures, the plastic of the 1/16" female luer fitting begins to melt, potentially sealing the flow opening of the luer fitting. Excess heat directly applied to silicone tubing was observed to cause point defects along the affected section of tubing when the tubing was pressurized, causing plastic deformation and failure of the tube. To avoid these complications, specific instructions have been outlined in **Appendix B – A Guide to Heat Shrink Tubing** for manufacturing these parts. Despite these limitations, heat shrink tubing is still the best option we tested for permanently sealing the silicone tubing to the syringe tip.

7. Silicone tubing sealing mechanism

We had a variety of options for sealing the free/open end of the silicone tubing to ensure pressurization. These included endcaps, and a variety of different clamps. Endcaps are small rubber plugs fitted to the distal end of the silicone tube and held permanently in place with glue. We considered pinch clamps for small diameter tubing due to their ease of use, low cost, and autoclavability.

The Ali MQP (2009) had a large variety of endcap designs including a needle endcap, blunt endcap, suture, and a combination of suture and a blunt endcap. The blunt endcap and needle endcap designs were multi-functional. Both aided in tissue ring loading by providing a point at the end of the silicone tube to place in the center of the tissue ring, from where you can push the ring up the silicone tube with forceps. The two endcaps also effectively sealed the silicone tubes from leaking due to air pressurization. Sealing the distal end of the tube may also pose a challenge in preloading the silicone tube with liquid for distension by syringe. The tube would have to be submerged completely in water or media to allow the air within the tube to be replaced with liquid before attaching the syringe. While these designs would have worked well in a vertical tissue chamber setting, they are far more difficult to accomplish in a horizontal setting. These may all seal the silicone tube effectively, but there is nothing present to hold the silicone tube taut and steady relative to the base of the tissue chamber. Clamping or sealing the endcap inside the tissue chamber would keep the tube taut.

Figure 25. Pinch clamp from World Precision Instruments (left) and Z-Man Corp (middle & right)

For this reason, our silicone tubing sealing mechanisms were narrowed down to various types of clamps. We chose to clamp the tubing inside of the tissue chamber in order to prevent complications that could arise from running the tube outside, such as media leakage due to the presence of extra outlets in the walls of the chamber. Clamps effectively seal tubing without leaks and simultaneously can hold the tubing taut and off the bottom of the tissue chamber, securing its position in 3D space. The first type of

clamp we tested was a pinch clamp from World Precision Instruments (**Figure 25, left**). This clamp was 100% leakproof based on pressurization testing involving distending silicone tubing with a syringe past 20%, yet lost much of its flexibility post-autoclaving. This led us to a patent pending model for a pinch clamp from Z-Man Corp (**Figure 25, middle and right**). These clamps are autoclavable, and more importantly, retain their functionality post-autoclaving. The pinch-to-open mechanism for this clamp is far more ergonomic than the previous clamp, eliminating the need to hold the clamp steady with a second hand during opening. The seal on the silicone tube is visibly much tighter than the previous clamp as well. The hole located in the base of these clamps also makes it possible for us to manufacture a peg or dowel and hole to mount the clamps within the tissue chamber. This would make the clamps removable and eliminate the need to permanently adhere the clamps to the chamber surface, which is helpful in the event of clamp malfunction or during sterilization of individual parts. The smaller ergonomic pinch clamp was chosen for our device due to size restrictions within the tissue chamber.

8. Tissue chamber materials

Our options for tissue chamber materials were limited by the fact that the material had to be biocompatible, autoclavable and/or sterilized by 70% ethanol, easily machinable, and relatively inexpensive. We needed to choose a type of plastic for our tissue chambers, base component of our design, removable tray, and tissue chamber lids.

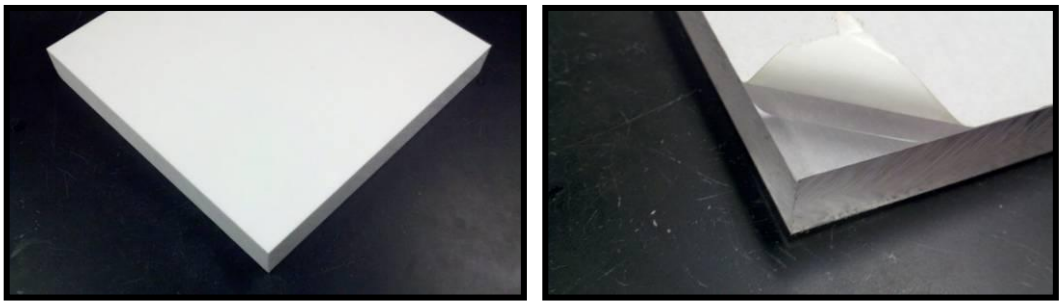


Figure 26. Ultra-high molecular weight polyethylene (left) and polycarbonate (right)

We decided to use ultra-high molecular weight polyethylene (UHMWPE) (**Figure 26, left**) for our tissue chambers due to its' high impact strength and durability, weather resistance (humid incubator environment), operating temperature range of -40^{o}F to 275^{o}F (250^{o}F needed for autoclaving), and that it meets ASTM (D4020, D4976, and D6712), FDA, and USDA specifications. Although this material does not

fulfill the need for transparency of the material to allow visualization of the sample within the chamber, transparent tissue chamber lids will satisfy this requirement.

We decided to use transparent abrasion-resistant polycarbonate (PC) (**Figure 26, right**) for the base and removable trays of our device. Based on client interviews, we discovered that the base and tray of our device need not be autoclaved between uses because they will not be in direct contact with tissue samples. It would merely need to be wiped down with 70% ethanol before entering the incubator. For this reason we chose abrasion-resistant polycarbonate to withstand harsh cleaners and solvents. PC also has high impact strength and durability, is weather resistant, and has an operating temperature range of -40^{o}F to 200^{o}F. Due to its low operating temperature, it cannot be sterilized within an autoclave (250^{o}F), and was thus not considered in designing tissue chambers.

Materials were selected based on several characteristics such as biocompatibility, sterilizability, size, ease of use, and how well they interface with other components in the device. Using these material choices, we designed and prototyped a device to mechanically condition tissue engineered blood vessels *in vitro*. The efficacy of individual materials and interfaces between design components were then tested through a series of qualitative and quantitative bench top experiments.

Chapter 5 – Design Verification

To validate specific features of our design we categorized the desired functions into two categories: those functions to be conducted by the tissue chamber and those to be accomplished by the mechanical conditioning system. The experiments used to determine the appropriate means for these features are explained in the following chapter.

5.1 Syringe Selection

We considered three different types of syringes that were readily available to use as a means of displacing specific volumes of water into the silicone tubing of our device to achieve 10 ± 1% distension.

The three syringes we examined were: sterile Monoject® 3mL luer-lock syringes, sterile 1mL luer-lock syringes, and sterile Monoject® 1mL "push-connect" or luer-slip syringes, all pictured in **Figure 27**. The volume of water required to achieve ~10% distension for a 10 cm long, 1.9578 mm inner diameter autoclaved silicone tube was found to be approximately 0.075 mL through extensive bench top testing of multiple samples. This volume was measured through observing the specific volume displaced by the 1 ml syringe that resulted in 10% distension of the outer diameter of the silicone tube. The experiment was performed by filling a 1 ml syringe with water completely, tapping all air bubbles out of the syringe barrel, and attaching two interlocking male and female luers. The female luer was heat shrunk to 10cm of silicone tubing (see Appendix # for manufacturing instructions). The system was flushed with water until the plunger read 0.3mL and clamped at its distal end with a pinch clamp. We used approximately 0.3 mL of water to preload the silicone tube and connectors prior to sealing the distal end of the tube and pressurizing it. Due to using such a small volume of water (0.3mL + 0.075mL + a safety factor of 0.225mL = 0.6mL), we limited our syringe options to between 1 and 3 mL syringes. The safety factor was used in the event that the user accidentally pushed the syringe plunger too hard and ejected too much water. It also allows for a larger cam to be used in our design further on, leaving room on the syringe barrel for larger volumes of water to be displaced by larger cam sizes in future studies.

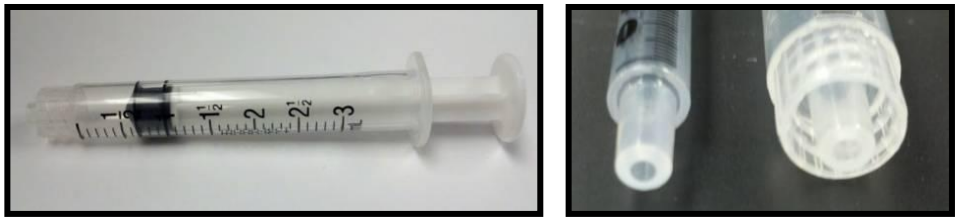


Figure 27. 3mL luer-lock syringe (left), Luer-slip vs. luer-lock syringe tips (right)

The 3mL Monoject® syringes came pre-packaged and sterilized. The barrel of the syringe was marked at 0.1mL increments, 0.1mL corresponding to 0.06 mm of space between graduations. The overall length of the syringe from luer-tip to plunger base is 80 mm when the syringe plunger is fully depressed, and 84.5 mm when at a pre-distension volume of 0.3mL, the safe minimum volume used for distension studies. It would require 1.5mm (0.075mL on a 3mL syringe ≈ 1.5mm) of syringe plunger movement to achieve ~10% distension in the attached silicone tubing. The tip of the syringe is fitted with a luer-lock which secures luer-based syringe tips to the syringe by twisting the detachable tips onto the barrel of the syringe. This ensures that the syringe tips remain attached during use and prevents the syringe tips from being pulled from the syringe when a force is applied.

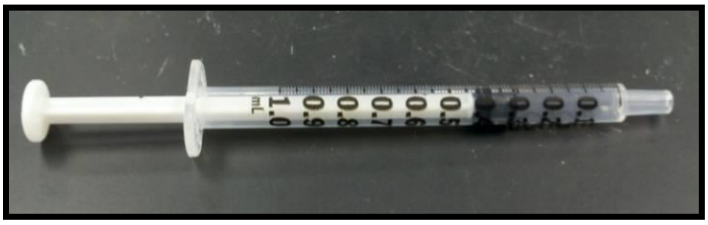


Figure 28. 1 mL push-connect or luer-slip syringe

The 1mL luer-lock syringe is identical to the 1mL "push-connect" or luer-slip syringe with the exception of the luer fitting at the tip of the syringe. A side-by-side comparison of luer-slip and luer-lock syringe tips can be seen in **Figure 28**. One mL luer-slip and luer-lock syringes come pre-packaged and sterilized. The barrels of these syringes are marked at 0.01mL increments, 0.01mL corresponding to ~0.017 mm of space between graduations. The overall length of the syringe from luer-tip to plunger base is 91.5 mm when the syringe plunger is fully depressed, and 109.5 mm when at a pre-distension volume of 0.3mL.

Further fatigue testing indicated that the shorter the syringe plunger during mechanical conditioning with the motor, cam and spring, the less likely the plunger is to bend and permanently deform over a

period of 2 days constantly running within an incubator. The safety factor was thus later changed to 0.125mL instead of 0.225mL so the plunger would travel from the 0.2mL point to 0.125mL instead of from 0.3mL to 0.225mL, decreasing the overall length of plunger left unsupported by the syringe barrel.

Due to these factors (summarized in **Table 4**), we decided to use a 1mL syringe in our design. The 4.5 mm (0.075mL on a 1mL syringe ≈ 4.5mm) distance required by the 1mL syringe to inject 0.075mL of water into the silicone tubing to achieve ~10% distension makes it far simpler to manufacture and operate the cam. It is more difficult to move a syringe by 1.5mm (3mL syringe) than by 4.5mm (1mL syringe). The larger working area over which to mechanically distend the silicone tubing is preferred, making it possible for us to use a larger cam if necessary. The 1mL syringe also has far smaller measurable volume increments along the barrel of the syringe, making it far simpler to measure small volume changes in the system. Between the luer-slip and luer-lock 1mL syringes, we decided to use a 1mL luer-slip syringe as seen in **Figure 28**. The 1mL luer-lock syringes we viewed for comparison in catalogs were bulkier at the tip than the luer-slip. The twist required to secure the luer-lock syringe tips into place was trivial compared to a push-connect tip. Locking luers were an unnecessary precaution that would add an additional complication to loading and preparing silicone tube samples during procedural set-up. 1mL luer-slip syringes are autoclavable, reusable, and inexpensive at roughly 17 cents per syringe.

Table 4. Summary of syringe characteristics

Syringe Type	Length of Syringe (Plunger Depressed)	Distance Traveled for 0.075mL Injection	Syringe Accuracy	Luer Attachment
1mL luer-slip	91.5 mm	4.5 mm	0.01 mL	Push
3mL luer-lock	80.0 mm	1.5 mm	0.1 mL	Push & Twist
1mL luer-lock	N/A	4.5 mm	0.01 mL	Push & Twist

5.2 Quantification of Displaced Volume

We tested our design alternatives to determine which approach was best suited at achieving 10% distension. Having decided upon a water-based pressurization system, rather than compressed air, we

needed to determine the volume of water required to distend a silicone tube. Specifically, our design called for a silicone tube 10 cm in length for optimal tissue sample loading.

5.2.1 Experimental Procedure

To measure the volume of water displaced by the 1 mL syringe that achieved the target magnitude of 10% distension, a water-filled syringe was used to statically inflate a tube with defined volumes in increments of 25 µL (**Figure 29Error! Reference source not found.**). Tubing diameters were obtained from still images of the inflated tubes using a Leica EZ4 D microscope and Leica Application Suite. For this experiment a total of 5 autoclaved samples were tested.

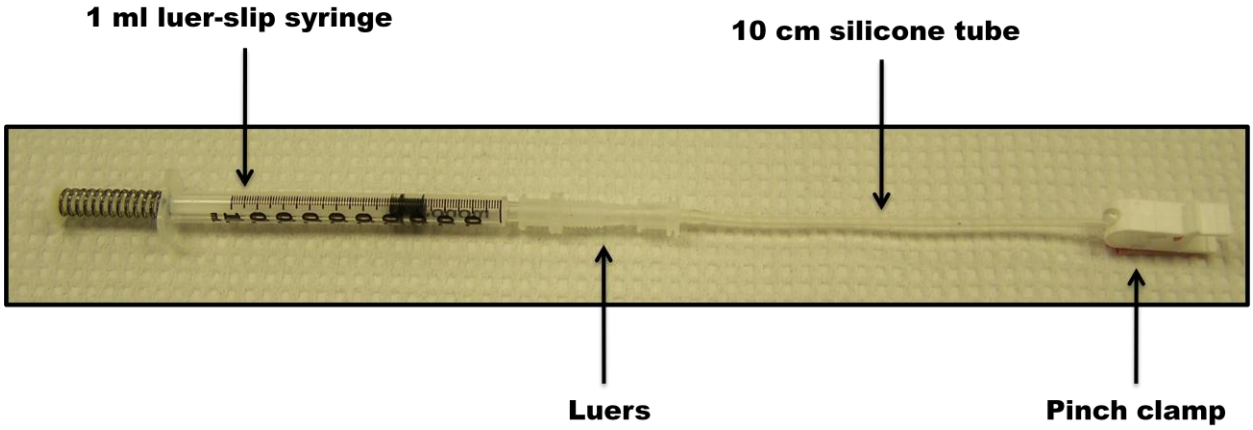


Figure 29. 1 mL luer-slip syringe connected to 1/16" barbed bayonet tip mated with a 1/16" barbed luer-lock tip that is attached by heat shrink tubing to a 10 cm silicone tube sample. A pinch clamp at the distal end of the tube was used as a means of fixation and sealing.

5.2.2 Experimental Results

In static inflation tests, 75 µl of water resulted in 9.80±0.23% distension of a 10 cm length of 1.96 outer diameter silicone tubing (**Figure 30Error! Reference source not found.**). Therefore, a 75 µL displacement volume was used for subsequent studies.

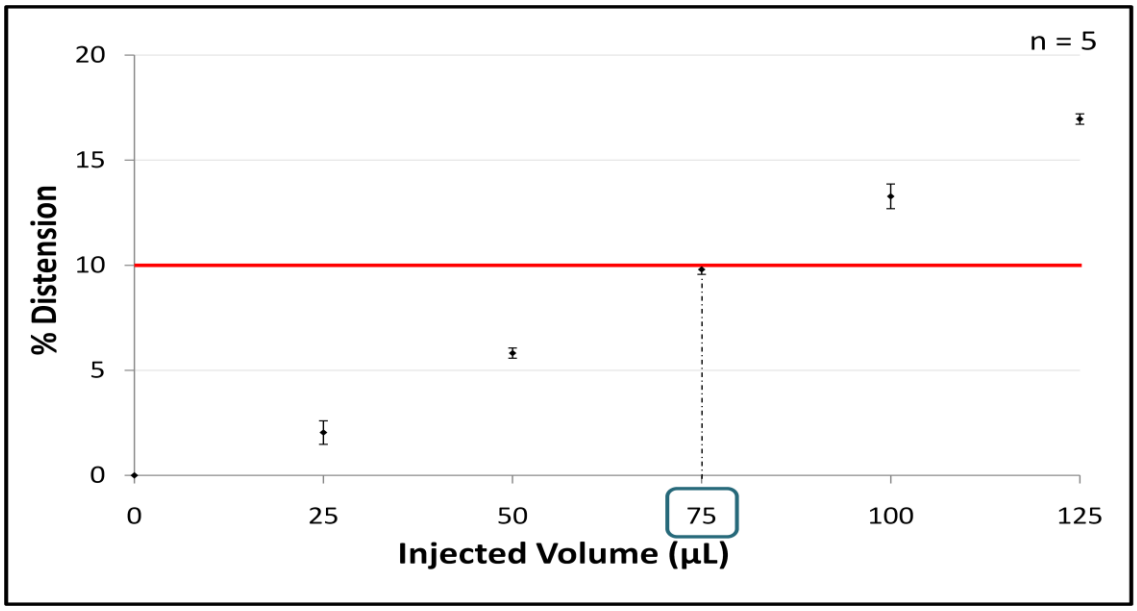


Figure 30. Required volume to achieve 10% distension of silicone tubing (n = 5; 9.8±0.23%). Red lines represent desired max and min distension values. Green line represents target value (10% distension).

5.3 Uniformity of Tubing Distension

The goal of this experiment was to determine how uniform the distension was along the length of the silicone tube. This allowed us to visualize the amount of working space for the placement of tissue rings.

5.3.1 Experimental Procedure

We marked each sample with nine markings (numbered 0-8) with 1 cm spacing between them (**Figure 31**). Then, similarly to the Quantification experiment, we statically distended the silicone tube by injecting 75 µL and measured the induced diameter by taking an image through the Leica Software. This was repeated for every reference point along the length of the tube.

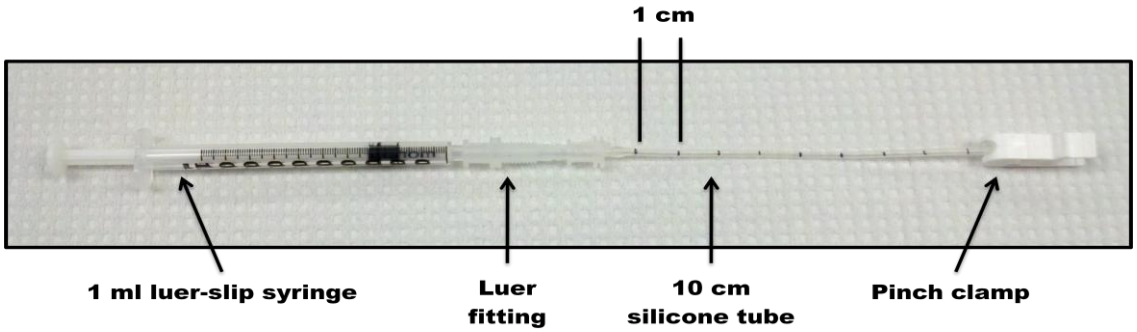


Figure 31. A 1 mL luer-slip syringe connected to a 1/16" barbed luer lock tip fixed to 10 cm silicone tube sample by heat shrink tubing. The 10 cm sample is marked with a black marker sealed and by a pinch clamp.

5.3.2 Experimental Results

Static inflation of autoclaved tubing samples (n=20) suggested that 10 ± 1% distension could achieved. Five reference points (points 2-6) were found to always be within 10±1% distension. After an injection of 75 µl of fluid, all points were within 10 ± 1.6% (Error! Reference source not found.**Figure 32**).

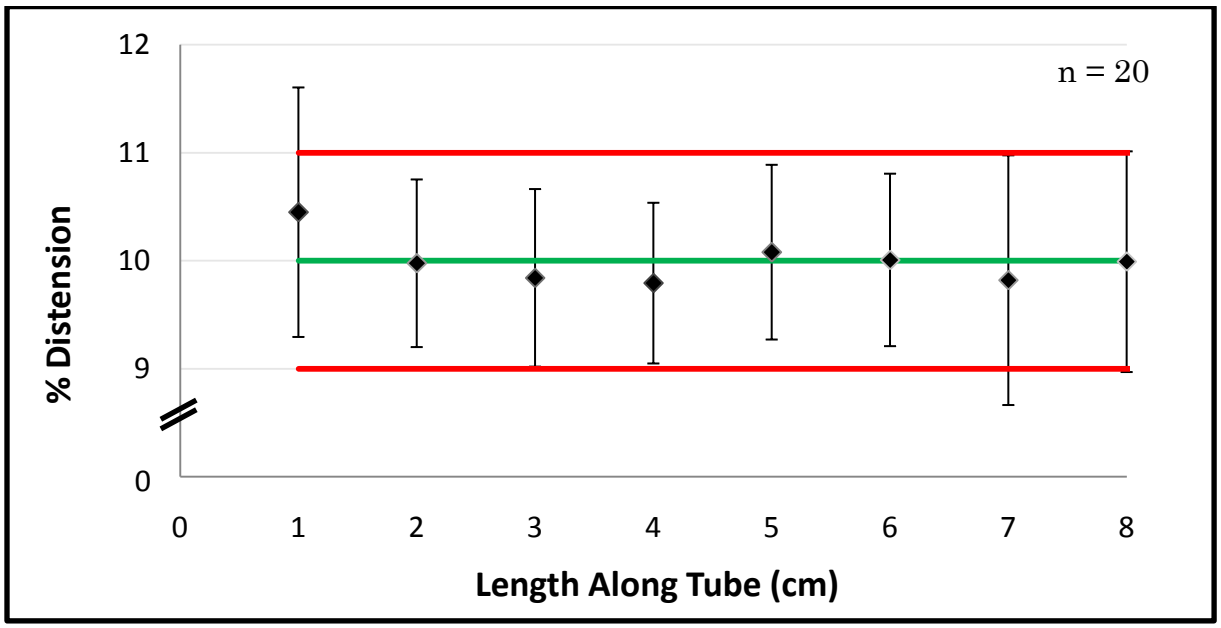


Figure 32. Uniform distension along silicone tubing (n=20). Red lines represent desired max and min distension values. Green line represents target distension (10%).

5.4 Tissue Ring Loading

5.4.1 Experimental Procedure

To verify the desired objectives of a high throughput and easy sample loading, four tubing samples were each loaded with 4-6 TEBV rings (**Figure 33**) and secured within our tissue chamber.

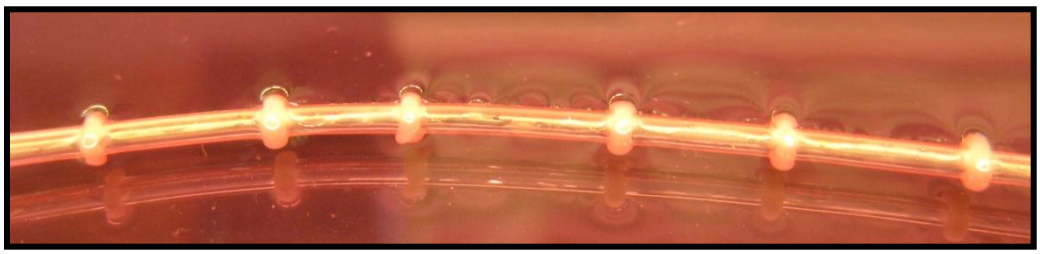


Figure 33. Six tissue rings loaded onto silicone tubing.

The chambers were filled with culture media and placed in the tissue chamber housing unit (**Figure 34**). For a complete procedure, see **Appendix E**.

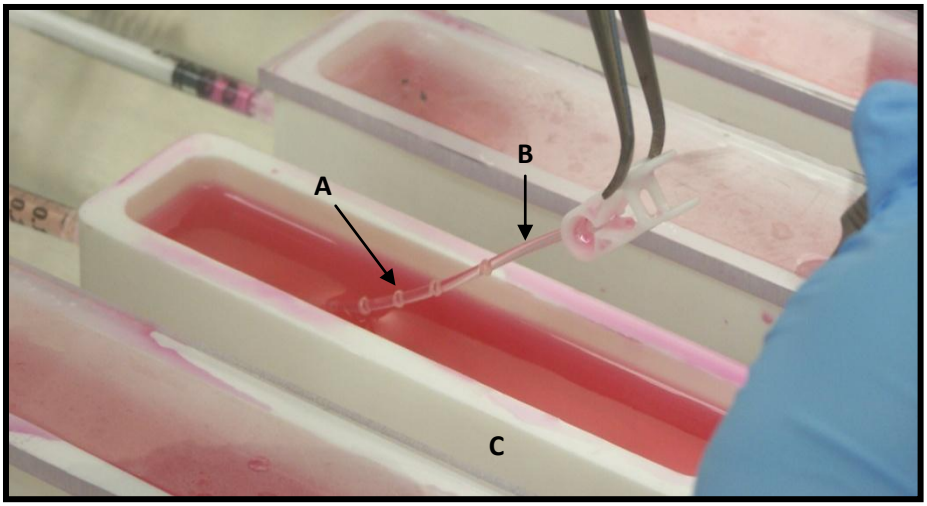


Figure 34. Tissue rings (A) mounted on silicone tubing (B) and loaded into the tissue chambers (C).

5.4.2 Experimental Results

Tissue ring samples (n=45) were successfully mounted (45/53; 85% success rate) onto 4 silicone tubes (n=4-6 per tube). After 3 and 7 day conditioning periods, samples remained viable and uncontaminated.

5.5 Preliminary Impacts of Mechanical Conditioning

5.5.1 Experimental Procedure

Using the experimental procedure outlined in **Appendix E**, four tissue chambers were prepared. In two of the four chambers, rotating cams were turned on to induce mechanical conditioning while the other two were left off and represented static culture control samples. The four chambers where then placed into the bioreactor system and run for two different time periods. One set, consisting of a static and conditioned chamber, was allowed to culture for 3 days while the other ran for a total of 7 days. To ensure that the cell constructs received proper nutrients, the media (DMEM, 1X with 4.5 g/L glucose, L-glutamine, & sodium pyruvate – modified with 10% FBS, 1% Penicillin/Streptomycin - 60 mL per chamber) was changed every two days. Following the end of the culture periods, the tissue rings were harvested for uniaxial tensile testing and histology to measure ultimate tensile stress (UTS), thickness, and cell density. Thickness was measured using DVT Imaging Software while UTS was

determined through the use of an Instron machine. The static samples were compared to the conditioned samples to determine the effectiveness of our device.

5.5.2 Experimental Results

Two individual trials were conducted, each consisting of two time points (3 and 7 day), in which both static (control) and mechanically conditioned (experimental) samples were examined for UTS, thickness, and cell density.

For the first trial (**Figure 35**) the average UTS of unconditioned samples after a three day culture period was 555.92 ± 129.78 kPa, whereas conditioned samples after 3 days were found to have a mean UTS of 429.60 ± 107.33 kPa ($p = 0.19$, therefore not statistically different). For the 7 day samples, unconditioned rings were found to have a UTS of 82.79 ± 13.29 kPa while the conditioned rings were measured to have a UTS of 122.42 ± 32.96 kPa ($p = 0.052$, therefore not statistically significant). The UTS for both static and conditioned ring samples were significantly stronger at 3 days compared to 7 days ($p<0.001$).

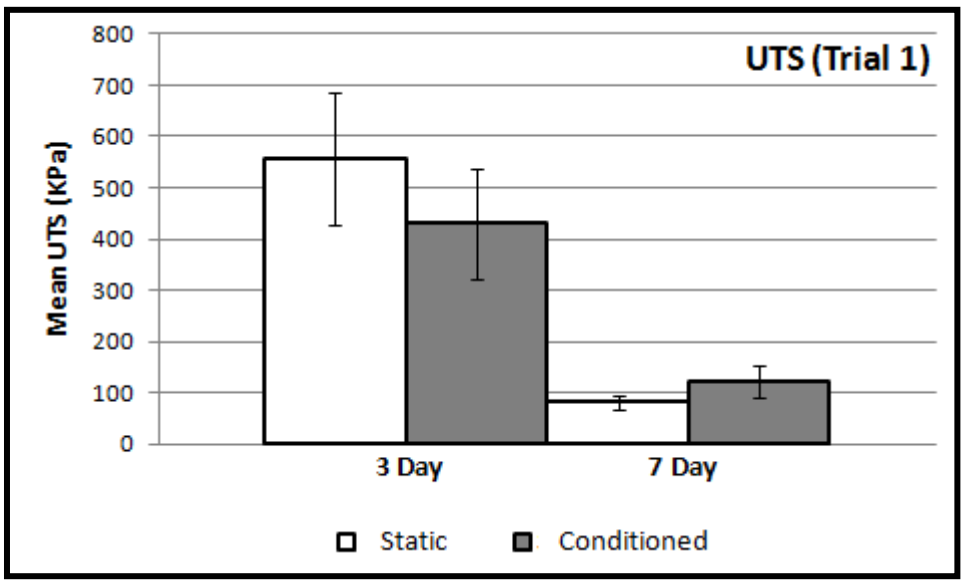


Figure 35. Ultimate tensile strength first trial. N-values are 2, 3, 2, and 4, respectively.

These calculations were repeated for the second trial (**Figure 35**). After three days of culture, the static samples exhibited an average UTS of 128.23 ± 36.8 kPa, whereas the conditioned samples were found be able to withstand 160.27 ± 31 kPa before failing ($p = 0.18$, therefore not statistically different). After seven days of culture, the static samples experienced a UTS of 84.19 ± 19.4 kPa compared to the

conditioned samples which had a mean UTS of 84.9 ± 8.9 kPa (p = 0.48, therefore not statistically different). All day 3 samples were compared to all day 7 samples; this statistical comparison yielded a p-value of 0.004, therefore confirming a statistical difference between the strengths of the samples. As before, the only conclusion that could be made was that the strength statistically decreased from 3 to 7 days. The p values for the other groupings were too low to determine statistical differences.

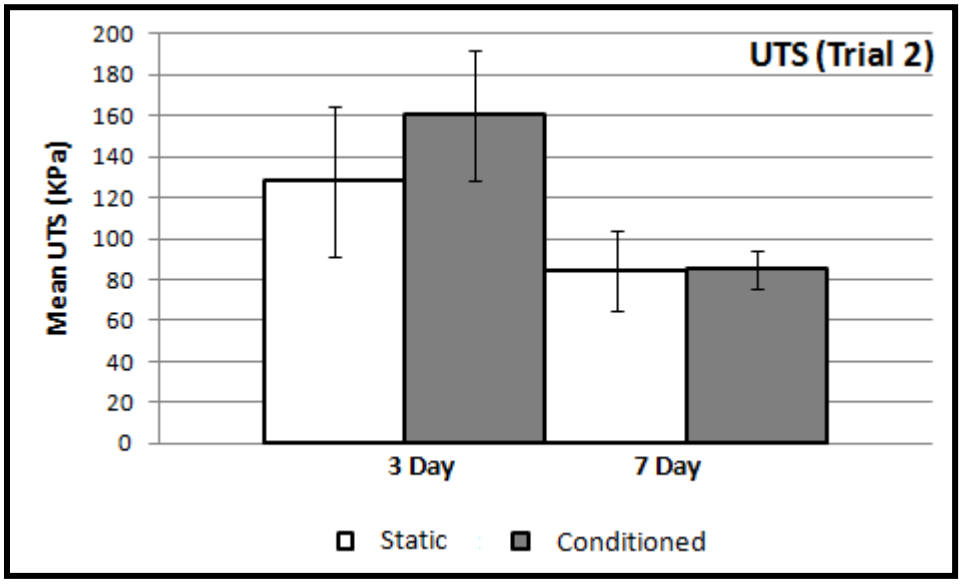


Figure 36. UTS second trial. N-values are 3, 3, 2, and 3, respectively.

In addition to UTS, we also measured the mean thickness of the unconditioned and conditioned samples. Displayed in **Figure 37** are the results from these experiments. The average thickness of static samples after 3 days in culture was 0.491 ± 0.069 mm, and the 3 day conditioned samples' average thickness was measured as 0.548 ± 0.045 mm (p = 0.16, therefore not statistically different). After 7 days in culture the static samples were found to have an average thickness of 1.045 ± 0.19 mm, whereas the conditioned rings had a mean thickness of 1.041 ± 0.078 mm (p = 0.47, therefore not statistically different). All day 3 samples were compared to all day 7 samples; this statistical comparison yielded a p-value of 3.43 E-6, therefore confirming a statistical difference between the thicknesses of the samples.

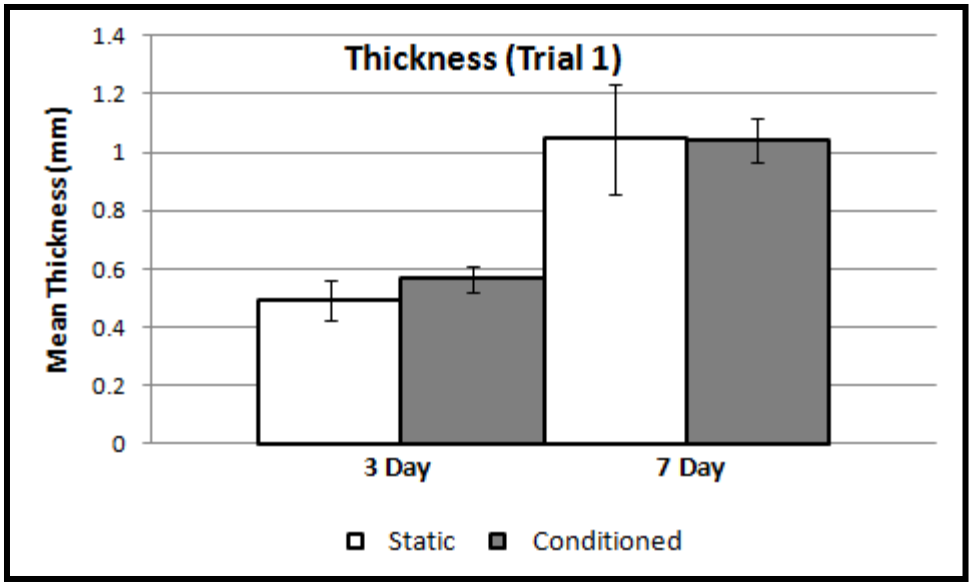


Figure 37. Thickness first trial. N-values are 2, 3, 2, and 4, respectively.

Thicknesses were also measured for the second trial (**Figure 38**). After three days of culture, the average thicknesses for static and conditioned samples were calculated to be 0.7018 ± 0.05 mm and 0.72 ± 0.02 mm, respectively (p = 0.20, therefore not statistically different). After being cultured for seven days, the thicknesses of the rings increased to 1.08 ± 0.08 mm for the static samples and 1.154 ± 0.09 mm for the conditioned samples (p = 0.10, therefore not statistically different). All day 3 samples were compared to all day 7 samples; this statistical comparison yielded a p-value of 9.6 E-10, again confirming a statistical difference between the thicknesses of the samples.

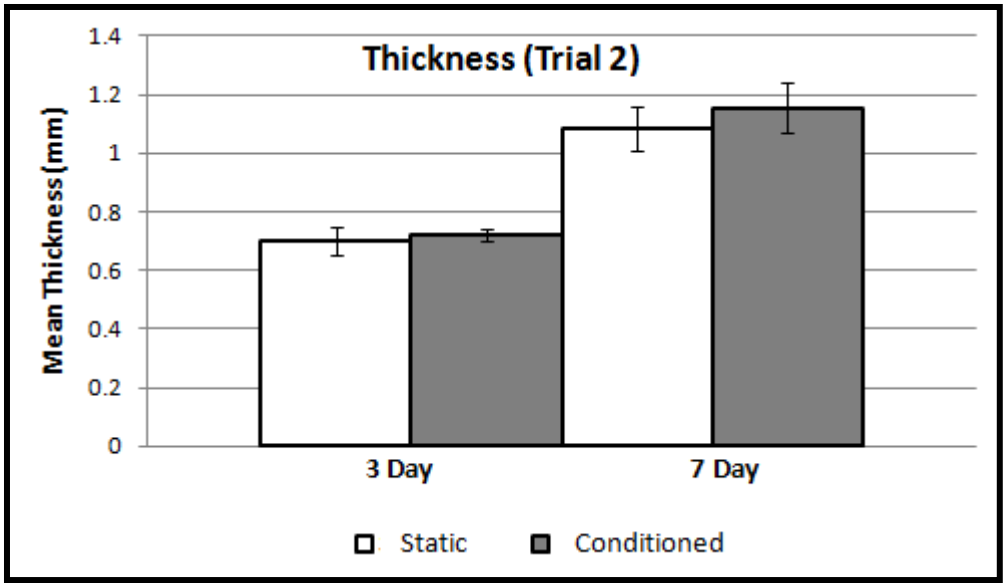


Figure 38. Thickness second trial. N-values are 5, 6, 6, and 5, respectively.

5.6 Histological Analysis

Rat aortic smooth muscle tissue rings (WKY 3M-22) were harvested at 3 and 7 days (**Figure 39**) for both sample sets.

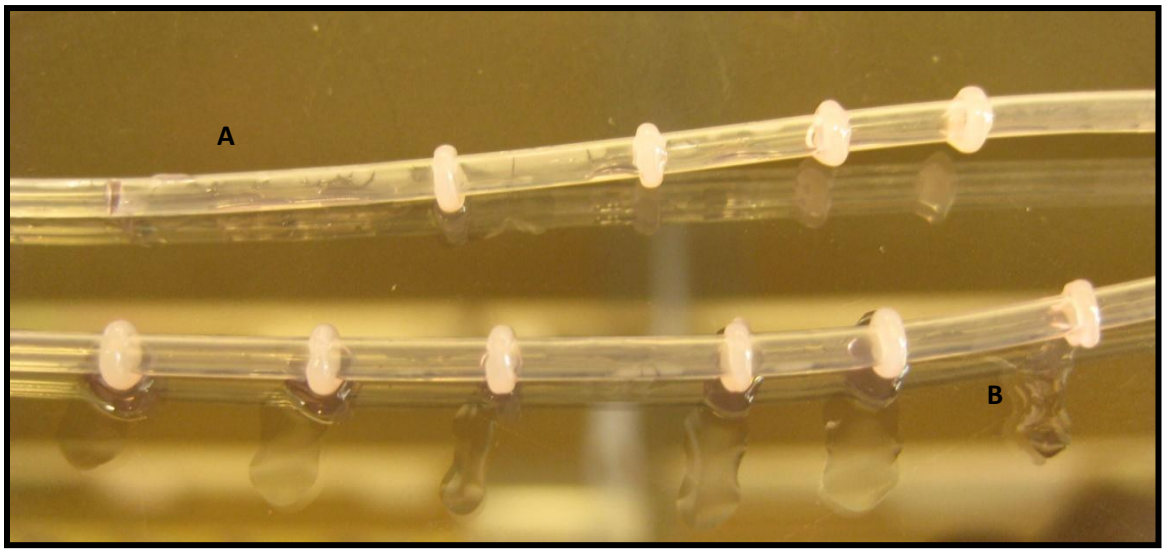


Figure 39. Conditioned (A) and static (B) tissue rings on silicone tubes after 7 days of culture.

The tissues were sectioned, mounted, and stained with hematoxylin and eosin and imaged under 20x magnification. Cell count and area of the tissue in square millimeters were calculated using ImageJ software. Mechanically conditioned samples yielded a density of 12.28 cells/mm^2 after 3 days **(Figure**

40- A) and 18.26 cells/mm^2 after 7 days **(B)**. Static samples yielded densities of 22.16 cells/mm^2 **(C)** and 15.87 cells/mm^2 **(D)** after 3 and 7 days, respectively. Data is based on sample sizes of 2 for each time period and culture condition with the exception of conditioned 7 days, which had 1 sample. This preliminary analysis suggests that cell density increased by 49% from three to seven days for conditioned samples, while static samples exhibited a decrease of 29% cell density according to ImageJ calculations.

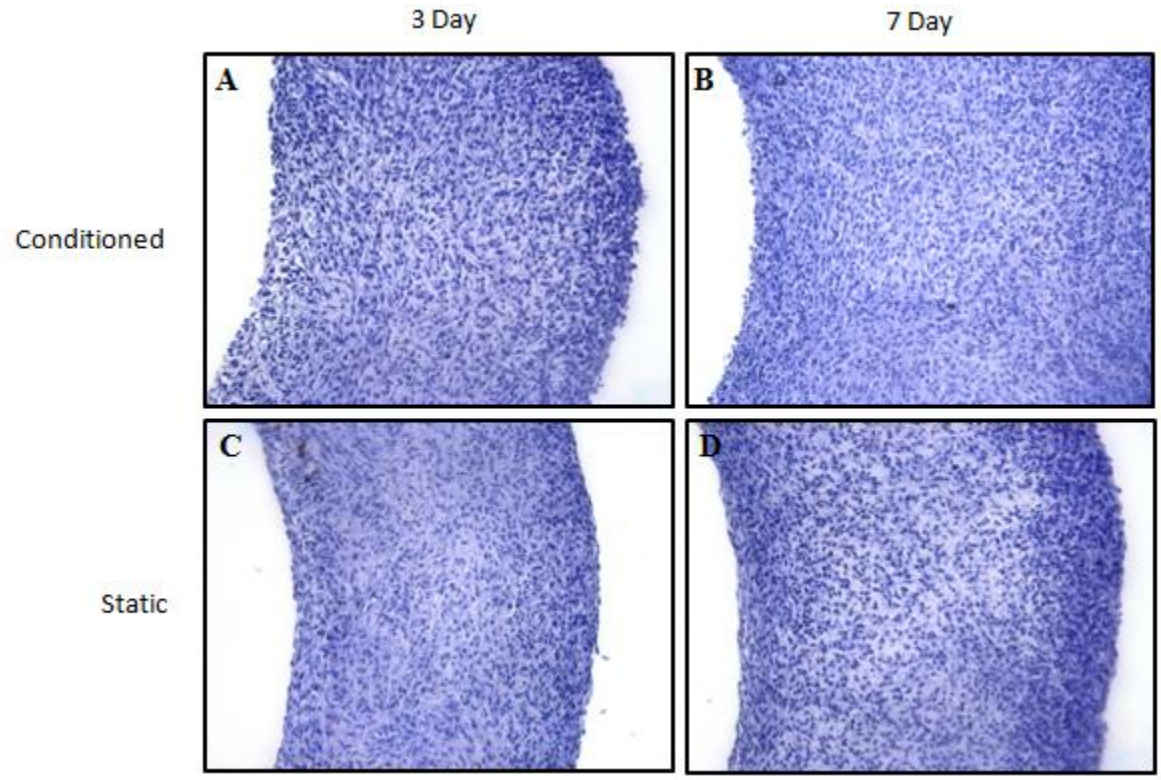


Figure 40. 3 and 7 day tissue ring samples stained with hematoxylin and eosin at 20x magnification.

Collagen staining was also conducted using Fast Green/Picrosirius Red for all tissue ring samples (**Figure 41**). Upon review under 20x and 40x magnification, little to no collagen appeared to be present. In order to definitively make a conclusion, the samples should be recut deeper into the tissue rings, or use a biochemical assay to quantify the amount of collagen in each ring sample.

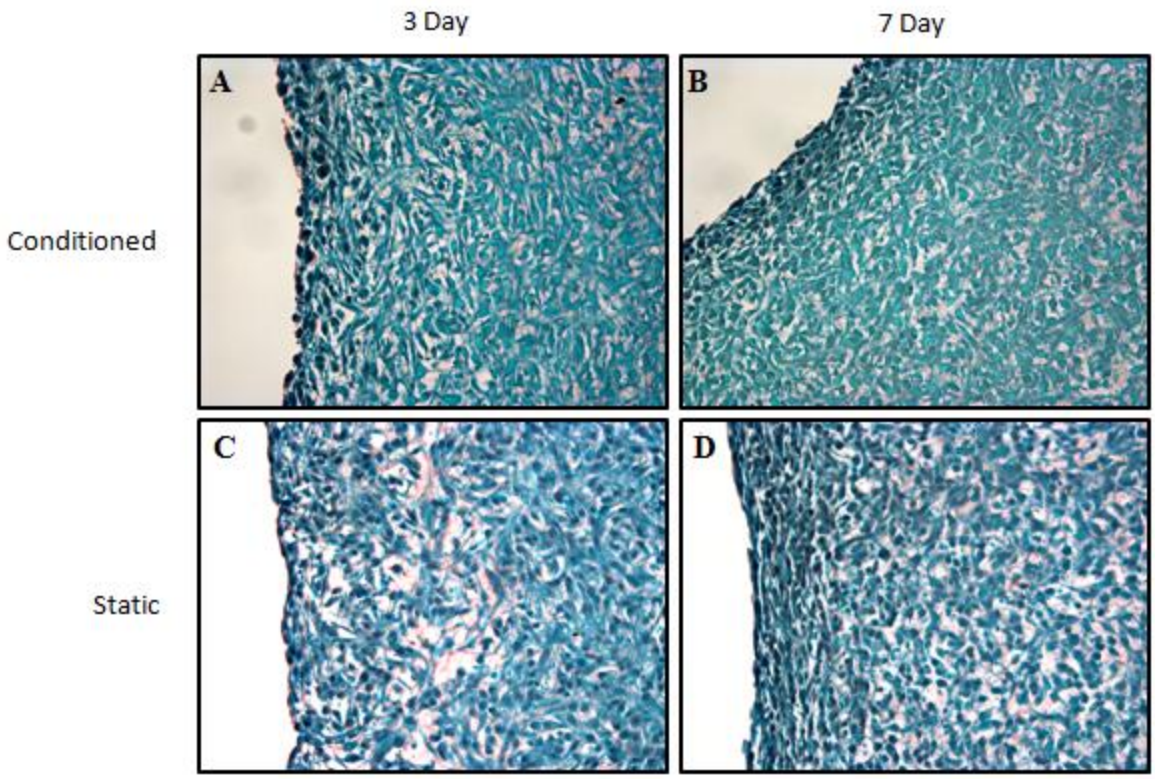


Figure 41. Tissue samples stained with Fast Green/Picrosirius Red at 40x magnification.

Overall, all tissue rings maintained a high cell density, remained viable, and were uncontaminated for all sample sets.

Chapter 6 - Discussion

Each component of the final assembly was designed to meet or help meet a project objective or constraint, including being easy to use and assemble, achieving the desired distension along the silicone tube, providing a sterile culture environment for tissue samples, and offering a number of customizable features to the user. Also, as our device promotes advances in medicine and patient wellness, it addresses several greater social and ethical concerns.

6.1 Bioreactor design

6.1.1. Successfully achieved 10±1% distension

The silicone tube was statically inflated and changes in outer diameter were measured in order to calculate the percent distension. It was determined that the displacement of 75µL of water by the 1 mL syringe would yield an average distension of 9.8 ± 0.2%.

6.1.2. Uniformity of distension leads to high throughput

The preloaded 10 cm silicone tube was marked at every centimeter and subjected to inflation of +75µL. While the tube exhibited inconsistent distension values at the proximal and distal ends, 10±1% distension was consistently achieved at markers 2-8. This suggested that the tube achieves uniform distension across at least 6 cm; thereby defining the length of usable tubing on which tissue rings may be loaded and conditioned. In a client interview, it was determined that six tissue samples could be loaded on each silicone tube. Therefore, the bioreactor may culture and condition a total of 30 tissue samples simultaneously.

6.1.3. Customizability

The bioreactor is designed so the user can run up to five isolated systems at one time. Each unit has its own mechanical conditioning system; the main components of this system are the motor and cam. The motors interface with a set of power switches so some motors may run at 60 RPM while others are shut off. This also presents the possibility of conditioning individual samples in intervals (e.g., one hour on, one hour off). The size of the cam may also be changed in order to adjust the volume of medium displaced by the syringe. A smaller cam will displace a smaller flow volume, and likewise a larger cam

will displace a larger volume. The individual tissue chambers provide isolated culture environments for each set of tissue rings. The user may adjust the type of culture media and additives in each system.

6.1.4. Device and materials are biocompatible

After 3 and 7 day culturing periods, cells remained viable and healthy without evidence of contamination. This is partially due to the biocompatible and sterilizable material choices (UHMWPE, sterile syringes, stainless steel dowels, polypropylene pinch clamps). Additionally, the tissue chamber's loose-fitting Petri dish-style lids allow gas exchange, and the removable tray allows the samples to be easily transported to a biosafety cabinet where cell culture media can be replenished. Finally, the bioreactor meets the defined size constraints and is able to fit in an incubator.

6.1.5. Easy to secure and remove samples

During the loading phase of cell testing, 88% (21 of 24) of tissue engineered blood vessel rings were successfully loaded onto the silicone tube. This confirms that the 10 cm silicone tube (OD=1.968, ID=1.9558) successfully houses the tissue rings and provides a simple, user-friendly assembly platform. Furthermore, the luer fittings allow the user to access the tubing and rings which facilitates the loading and unloading of culture samples into and out of the tissue chambers.

6.1.6. Time and budget met

The entire bioreactor was designed and manufactured within the 21 week time constraint, and the team produced a working prototype for less than the $368 budget.

6.2 Economic impact

This bioreactor design has the potential for economic impacts in the research community and in clinical use. The bioreactor was manufactured for less than $400. For comparison, the Harvard 22 Syringe Pump which uses two syringes compared to five and may only contribute as a single component of other bioreactor designs is advertised as the industry standard and costs over $3000 (Harvard Pump 22, 2010).

The production of our high-throughput bioreactor would supplement the production rate of cell culture and research. This may lead to earlier FDA approved TEBV grafts that could be used to treat cardiovascular disease. As these types of grafts would consist of tissues that are derived from the

patient, fewer instances of rejection can be expected. This will ultimately result in the user enduring fewer costs associated with pharmaceuticals, follow-up procedures, and hospital stays.

6.3 Environmental impact

The bioreactor is composed primarily of durable plastics that are abrasion resistant and may be sterilized in an autoclave, with the only exceptions being bulk products such as pinch clamps and luer fittings. The use of sterilizable, reusable materials minimizes the waste production associated with the device.

6.4 Societal influence

According to the World Health Organization (WHO, 2010), heart disease is the second leading cause of death in the world, killing about 2.5 million people annually. The development of patient-derived TEBV grafts could therefore have a positive impact on a tremendous number of patients worldwide.

6.5 Ethical concerns

The use of laboratory animals as models for experimental purposes is a common topic of debate. Tissue engineered constructs grown *in vitro* offer an alternative to animal models during research and development. Furthermore, this device and all relevant research may lead to saving lives and improving the conditions of millions of patients.

6.6 Health and safety issues

Our bioreactor facilitates research that may develop usable, patient-derived blood vessel grafts. Self-derived grafts are generally safer than synthetic grafts or donor transplants, where mechanical failure, infection, compliance mismatches, and rejection of the graft are prominent issues. The production of this bioreactor and the resultant TEBVs will ultimately lead to advances in the medical industry and improved patient care.

6.7 Manufacturability

The bioreactor primarily consists of components that can be machined using high-speed milling techniques and a series of machining files. Most other components in the bioreactor are standard parts

that can be purchased from catalogs, such as luer fittings, silicone tubing, and 12V hobby motors. Minimal assembly is required by the user, and all assembly only requires simple common tools such as a screwdriver.

6.8 Sustainability

The plastics that are used in the body of the bioreactor (UHMWPE and polycarbonate) are both recyclable materials. Machining scraps and even the device itself can be reprocessed and used for other applications, thereby minimizing the amount of waste produced in the production and use of the bioreactor.

Chapter 7 – Final Design & Validation

A computer-generated drawing of the final design assembly is shown in **Figure 42**. A photograph of the final design assembly is show in **Figure 43**.

The primary architecture of the assembly consists of a polycarbonate base (**Figure 42** & **Figure 43 - 1**). The surface of the base contains four corner barriers (**2**) to hold a polycarbonate tray (**3**). The tray has been machined to contain five wells in which milled ultra high molecular weight polyethylene (UHMWPE) tissue chambers sit (**4**). Each chamber houses a series of threaded luer fittings that are fitted with a 10 cm, 1.968 mm outer diameter silicone tube. The distal end of the tube is clamped shut using a polypropylene pinch clamp, which is secured to the bottom of the chamber using a 1/8" stainless steel dowel (see **Section 4.2.2 - 3**).

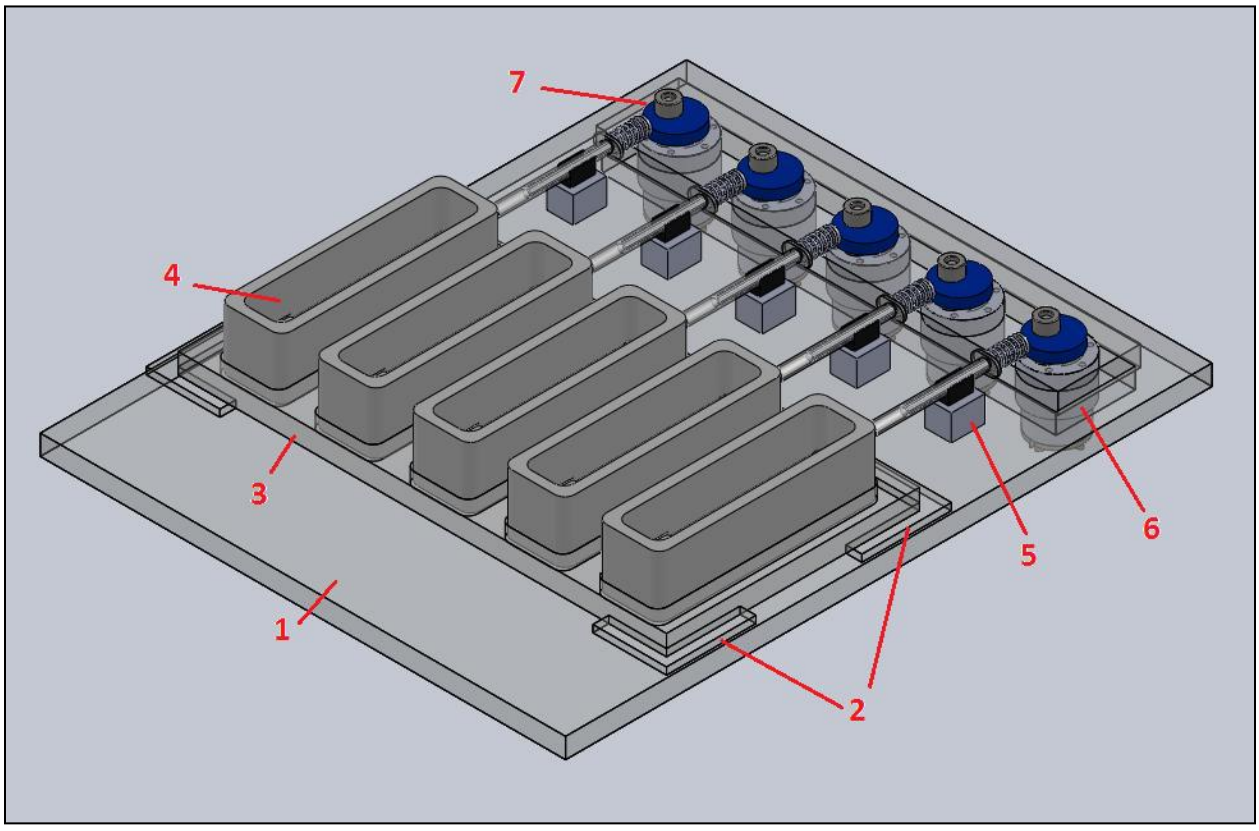


Figure 42. Final design (CAD)

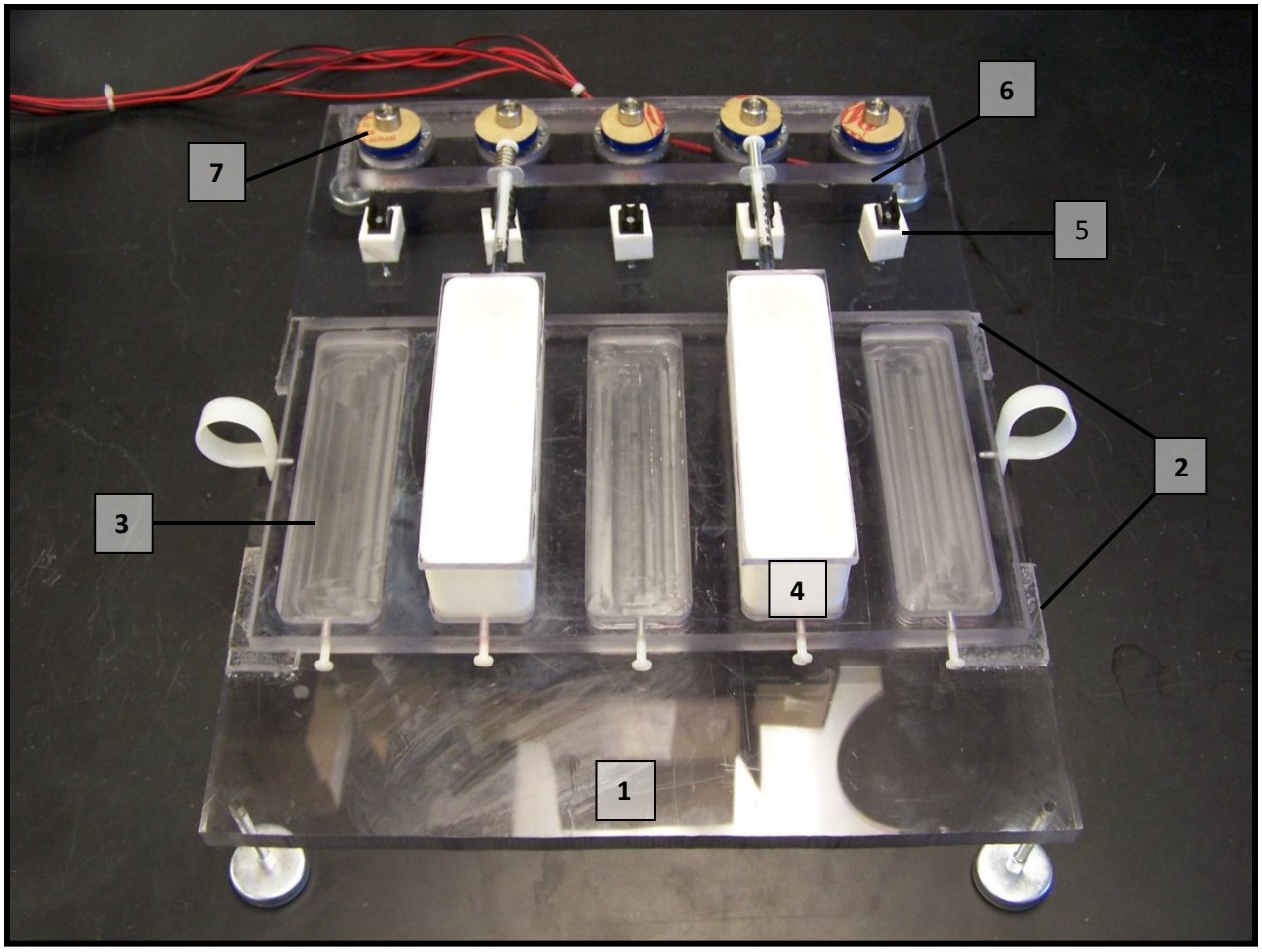


Figure 43. Final design

The base also contains five hose clamps (**5**) and a milled section in which five 12V 60 RPM hobby motors are mounted (**6**). Each motor contains a cam and interfaces with a reverse-engineered 1 mL syringe pump (**7**) as described in **4.1.1-4**. The design's mechanical conditioning component consists of five 12 volt, 60 RPM gear-box hobby motor (**Figure 42** & **Figure 43Error! Reference source not found. - 8**) which s fixed with a circular cam (**9**). As the cam rotates, it depresses the plunger of the fixed 1-mL syringe (**10**). It has been determined that the distance the plunger needs to be depressed in order to displace enough water to provide a 10% distension in the silicone tubing is 4.5 mm. Therefore, the axis of rotation for the cam is offset from the center by 4.5 mm.

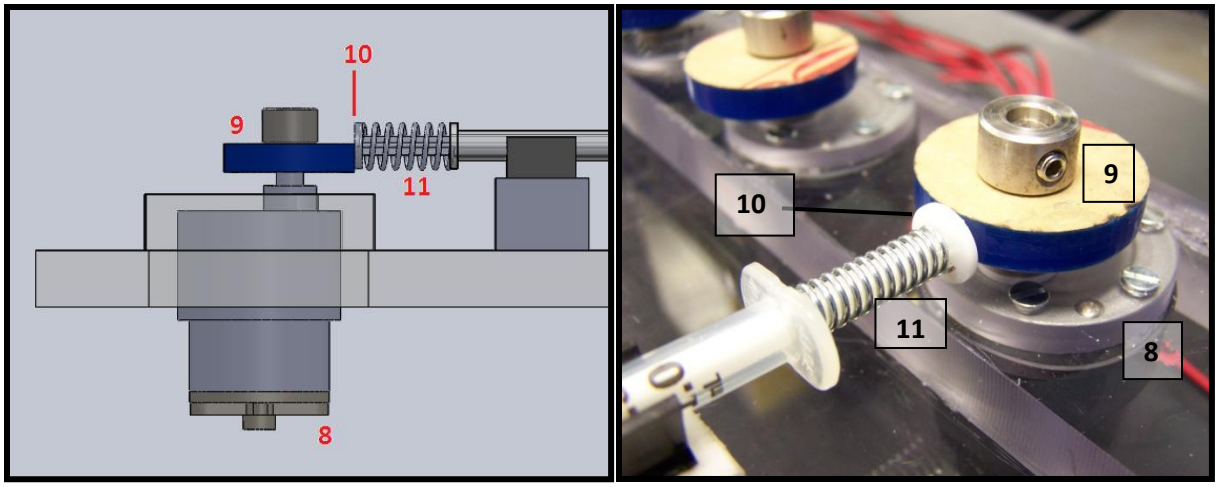


Figure 44. Mechanical stimulation.

Between the plunger head and the syringe body is a spring (**11**) which allows the plunger to retract as it is in contact with the short leg of the cam. This essentially creates a syringe pump. When interfaced with the silicone tubing through the luer fittings in the new tissue chamber design, the displacement of the syringe plunger will cause the silicone tubing in the tissue chamber to distend (the amount of distension depends on the size of the cam).

Both the tray and the base can be manufactured using high speed milling techniques. This assembly was chosen as the final design because its materials and architecture helps maintain the stability of the assembly. Also, the base internalizes most of the mechanical components in the design. Furthermore, the removable tray provides a definable housing unit for the tissue chambers and allows the user to easily remove and transport all tissue chambers at once.

These design aspects are appealing to both the designer and user because the small, individualized motors minimize the size of the entire design while allowing for customizability and individual shutoffs. Also, by having isolated a single mechanical failure is not likely to compromise the whole system. The removals of the wheel and flat arm are also expected to decrease failure rates as there are fewer mechanical interfaces in the system. Finally, this component of the design allows for increased customizability; while the cam has been designed to displace only enough water to distend the silicone tubing (and therefore the TEBV samples) by 10%, this cam may simply be changed out with another of a larger diameter to provide greater distension of the tubing.

The final design component is the tissue chamber compartment. This design is preferred to the other design alternatives because it completely separates the silicone tubing and pinch clamping from the mechanical system and the environment, and therefore significantly reduces the risk of contamination. Also, the use of the luer fittings allows for easy removal and attachment of silicone tubes and TEBV samples.

Our device offers several unique features that overcome the limitations of current devices. Our device offers high throughput options by allowing for the conditioning of up to 30 tissue rings simultaneously. The device has also been designed so the cam, syringe, and silicone tubing can be removed and replaced with different sized components in order to adjust the flow volume and frequency, and to accommodate different sized tissue rings. Tissue chambers are isolated from one another and the motors run independently. This allows several different culture conditions and experimental variables to be altered during a single experiment. Standard replaceable parts have been incorporated into the design in order to allow the user to easily replace damaged or contaminated components. Internalizing the motors in the polycarbonate base helps align and secure several design components. Internalized motors keep mechanical systems separate from tissue chambers so they may remain within the incubator while the tissue samples are transported for feeding using the removable tray.

Chapter 8 – Conclusions and Recommendations

Through component verification and bench top tests, both aspects of the bioreactor were validated: the mechanical conditioning system and the tissue chamber. This validation is proof-of-concept suggesting that the bioreactor could be used to successfully impart mechanical conditioning on tissue engineered ring samples.

8.1 Design Features

The design of a novel bioreactor which cyclically imparts mechanical conditioning was accomplished. The preliminary tests in conjunction with the bench top validations lead to the conclusion that a 10 cm long silicone tube sample could uniformly be distended by ~10% and therefore can impose cyclic circumferential strain on tissue engineering ring constructs.

Compared to other devices currently available, our bioreactor design is beneficial because it allows for a great deal of variability and customizable features. Changes in culture conditions can be varied by altering the dimensions of the easily replaceable cam component of the mechanical stimulation system. Additionally the individual tissue chambers allow several culture media conditions to be altered at once. Motors run independently from one another so several static and mechanically conditioned samples can be cultured simultaneously and for different periods of time.

Another benefit to our device is that with the total number of tissue chambers, a high throughput is achieved. Our device is capable of housing up to six tissue rings per chamber and the entire system is capable of maintaining five chambers yielding in total 30 tissue rings being conditioned *in vitro* during a single experiment.

8.2 Future Recommendations

Having conducted one successful full system experiment the team has developed the following recommendations.

The current hose clamps being used as syringe holders are very stiff and have been shown to crack over time with repeated use. We suggest that an alternative be designed or purchased. Even though the hose clamps successfully support the syringe during the conditioning cycles, it is very difficult to insert and

remove the syringe barrels. Metal syringe holders would be an appropriate alternative to the current holders. Additionally, the syringe plunger was found to be prone to deformation due to the high levels of shear stress created at the plunger and cam interface. We suggest that either a metal syringe or a stiffer syringe be used.

Small amounts of leaking were also observed in two of the four chambers during testing. It was determined that an adjustment of the threaded luer located in the wall of the tissue chamber resolved this issue. However, in order to avoid this from occurring during future experiments we suggest that a rubber o-ring be placed around the thread of the luer. Another alternative could be to permanently fix the threaded luer into the wall of the tissue chamber, preventing the possibility of user error during assembly.

Even though the team experienced no contamination during our week long experiment, we suggest that tissue chamber lids be machined with overhang rather than the current design's lids which simply rest on top of the tissue chambers. We feel that this will aid in the prevention of spilling during transport from biosafety cabinet to incubator and will further reduce the chance of contamination.

During the manufacturing stage we learned that the UHMWPE sheets used for the tissue chambers was a relatively soft plastic compared to other materials available in the market. For our purposes, the material was sufficient, however it was not the easiest to machine due its softness. We recommend that alternative plastics be considered to avoid issues with milling, tapping, and cutting.

References

Ali, H., Breindel, J., Bullock, S., & Herrera, J. (2009). *Bioreactor to Dynamically Condition Tissue-Engineered Vasculature.* (MQP Worcester, MA: Worcester Polytechnic Institute. Retrieved from http://gordonlibrary.wpi.edu/vwebv/holdingsInfo?bibId=298689

Atala, A., Lanza, R., Nerem, R., & Thomson, J. (2008). *Principles of Regenerative Medicine* . Burlington [Mass.]: Elsevier Academic Press.

Ballyns, J. J., & Bonassar, L. J. (2011). Dynamic Compressive Loading of Image-Guided Tissue Engineered Meniscal Constructs. *Journal of Biomechanics, 44*(3), 509.

Bourne, P., Favreau, J., Mawhiney, K., & Ortiz, A. (2010). *Design and Testing of a Novel Cell Seeding and Bioreactor System for the In-Vitro Growth of Scaffold-Free Tissue Tubes for Tissue Engineered Blood Vessels.* (MQP Worcester, MA: Worcester Polytechnic Institute. Retrieved from http://gordonlibrary.wpi.edu/vwebv/holdingsInfo?bibId=678959

Buschmann, M. D., Gluzband, Y. A., Grodzinsky, A. J., & Hunziker, E. B. (1995). Mechanical Compression Modulates Matrix Biosynthesis in Chondrycyte/Agarose Culture. *Journal of Cellular Science, 108*, 1497.

Butler, D. L., Goldstein, S. A., Guldberg, R. E., Guo, X. E., Kamm, R., Laurencin, C. T., Tranquillo, R. T. (2009). The Impact of Biomechanics in Tissue Engineering and Regenerative Medicine. *Tissue Engineering.Part B, Reviews, 15*(4), 477-484. doi:10.1089/ten.TEB.2009.0340

Cheng, G. C., Briggs, W. H., Gerson, D. S., Libby, P., Grodzinsky, A. J., Gray, M. L., & Lee, R. T. (1997). Mechanical Strain Tightly Controls Fibroblast Growth Factor-2 Release from Cultured Human Vascular Smooth Muscle Cells. *Circulation Research, 80*(1), 28.

Chiquet, M., Matthisson, M., Koch, M., Tannheimer, M., & Chiquet-Ehrismann, R. (1996). Regulation of Extracellular Matrix Synthesis by Mechanical Stress. *Biochemistry and Cell Biology, 74*(6), 737.

Dartsch, P. C., & Hammerle, H. (1986). Orientation Response of Arterial Smooth Muscle Cells to Mechanical Stimulation. *European Journal of Cell Biology, 41*(2), 339-346.

DeLoach, S. S., & Townsend, R. R. (2008). Vascular Stiffness: Its Measurement and Significance for Epidemiologic and Outcome Studies. *Clinical Journal of the American Society of Nephrology, 3*(1), 184-192. doi:10.2215/CJN.03340807

Diamantouros, S., Flanagan, T. C., Finocchiaro, T., Deichmann, T., Wilhelmi, M., Schmitz-Rode, T., & Jockenhoevel, S. (2009). Design of a Bioreactor System for the Mechanical Conditioning of Small Diameter Tissue-Engineered Vascular Grafts. *International Journal of Artificial Organs, 32*(7), 458.

Diamantouros, S. E., Flanagan, T. C., Finocchiaro, T., Deichmann, T., Wilhelmi, M., Schmitz-Rode, T., & Jockenhoevel, S. (2009). Development of a System for the Performance and Evaluation of Mechanical Conditioning on Tissue Engineered Vascular Grafts. *10,* 146 -149. doi:10.1007/978-3-642-03900-3_43

Dobson, J., Cartmell, S. H., Keramane, A., & El Haj, A. J. (2006). Principles and Design of a Novel Magnetic Force Mechanical Conditioning Bioreactor for Tissue Engineering, Stem Cell Conditioning, and Dynamic in Vitro Screening. *IEEE Tranactions on Nanobioscience, 5*(3), 173-177.

Electric Solenoid Actuators. Retrieved 12/13/2010, 2010, from http://www.tpub.com/content/doe/h1013v2/css/h1013v2_166.htm

Ferrechio, L., Lopez, A., Skelly, J., & Thompson, J. (2009). *Bioreactor Design for Vascular Tissue Engineering.* (MQP Worcester, MA: Worcester Polytechnic Institute. Retrieved from http://gordonlibrary.wpi.edu/vwebv/holdingsInfo?bibId=298751

Freed, L. E., Guilak, F., Guo, X. E., Gray, M. L., Tranquillo, R., Holmes, J. W., Vunjak-Novakovic, G. (2006). Advanced Tools for Tissue Engineering: Scaffolds, Bioreactors, and Signaling. *Tissue Engineering, 12*(12), 3285-3305. doi:10.1089/ten.2006.12.3285

Fujiwara, K. (2003). Mechanical Stresses Keep Endothelial Cells Healthy: Beneficial Effects of a Physiological Level of Cyclic Stretch on Endothelial Barrier Function. *American Journal of Physiology;Lung Cellular and Molecular Physiology, 285*(4), L782-4. doi:10.1152/ajplung.00142.2003

Gassman, A. A., Kuprys, T., Ucuzian, A. A., Brey, E., Matsumura, A., Pang, Y., Greisler, H. P. (2010). Three-Dimensional 10% Cyclic Strain Reduces Bovine Aortic Endothelial Cell Angiogenic Sprout Length and Augments Tubulogenesis in Tubular Fibrin Hydrogels. *Journal of Tissue Engineering and Regenerative Medicine,* doi:10.1002/term.323

Gimbronejr, M., Andreson, K., & Topper, J. (1999). The Critical Role of Mechanical Forces in Blood Vessel Development, Physiology and Pathology. *Journal of Vascular Surgery, 29*(6), 1104-1151. doi:10.1016/S0741-5214(99)70252-1

Goldman, S., Zadina, K., Ovitt, T., Copeland, J., Krasnicka, B., & Anderson, R. (2004). Long-Term Patency of Saphenous Vein and Left Internal Mammary Artery Grafts After Coronary Artery Bypass Surgery: Results from a Department of Veterans Affairs Cooperative Study *44*(11), 2149.

Guilak, F., Sah, R. L., & Setton, L. A. (1997). Physical Regulation of Cartilage Metabolism. In V. C. Mow, & W. C. Hayes (Eds.), *Basic orthopaedic biomechanics* (2nd ed., pp. 179) Lippincott-Raven.

Gwyther, T. A., Hu, J. Z., Christakis, A. G., Skorinko, J. K., Shaw, S. M., Billiar, K. L., & Rolle, M. W. (2011). Engineered Vascular Tissue Fabricated from Aggregated Smooth Muscle Cells. *Cells Tissues Organs,* doi:10.1159/000322554

Hahn, M. S., McHale, M. K., Wang, E., Schmedlen, R. H., & West, J. L. (2007). Physiological Pulsatile Flow Bioreactor Conditioning of Poly(ethyleneglycol)-Based Tissue Engineered Vascular Grafts. *Annals of Biomedical Engineering, 35*, 190.

Harvard Pump 22. (2010). Retrieved 2/15, 2010, from http://www.instechlabs.com/Pumps/syringe/22.php

Huynh, T. N., & Tranquillo, R. T. (2010). Fusion of Concentrically Layered Tubular Tissue Constructs Increases Burst Strength. *Annals of Biomedical Engineering, 38*(6), 2226. doi:10.1007/s10439-010-0045-z

Isenberg, B., & Wong, J. (2006). Building Structure Into Engineered Tissues. *Materials Today, 9*(12), 54-60. doi:10.1016/S1369-7021(06)71743-6

Isenberg, B. C., & Tranquillo, R. T. (2003). Long-Term Cyclic Distention Enhances the Mechanical Properties of Collagen-Based Media-Equivalents. *Annals of Biomedical Engineering, 31*(8), 937-949. doi:10.1114/1.1590662

Kanda, K., Matsuda, T., & Oka, T. (1993). Mechanical Stress Induced Cellular Orientation and Phenotypic Modulation of 3-D Cultured Smooth Muscle Cells. *American Society for Artificial Internal Organs, 39*(3), 686.

Kelm, J. M., Lorber, V., Snedeker, J. G., Schmidt, D., Broggini-Tenzer, A., Weisstanner, M., Hoerstrup, S. P. (2010). A Novel Concept for Scaffold-Free Vessel Tissue Engineering: Self-Assembly of Microtissue Building Blocks. *Journal of Biotechnology, 148*(1), 46-55.

Kim, B. S., Nikolovski, J., Bonadio, J., & Mooney, D. J. (1999). Cyclic Mechanical Strain Regulates the Development of Engineered Smooth Muscle Tissue. *Nature Biotechnology, 17*(10), 979.

Kolpakov, V., Rekhter, M. D., Gordon, D., Wang, W. H., & Kulik, T. J. (1995). Effect of Mechanical Forces on Growth and Matrix Protein Synthesis in the In-Vitro Pulmonary Artery: Analysis of the Role of Individual Cell Types. *Circulation Research, 77*(4), 823-831.

Konig, G., McAllister, T. N., Dusserre, N., Garrido, S. A., Iyican, C., Marini, A., L'Heureux, N. (2009). Mechanical Properties of Completely Autologous Human Tissue Engineered Blood Vessels Compared to Human Saphenous Vein and Mammary Artery. *Biomaterials, 30*(8), 1542-1550. doi:10.1016/j.biomaterials.2008.11.011

Ku, C. H., Johnson, P. H., Batten, P., Sarathchandra, P., Chambers, R. C., Taylor, P. M., Chester, A. H. (2006). Collagen Synthesis by Mesenchymal Stem Cells and Aortic Valve Interstitial Cells in Response to Mechanical Stretch. *Cardiovascular Research, 71*(3), 548-556. doi:10.1016/j.cardiores.2006.03.022

Kulik, T. J., & Alvarado, S. P. (1993). Effect of Stretch on Growth and Collagen Synthesis in Cultured Rat and Lamb Pulmonary Arterial Smooth Muscle Cells. *Journal of Cellular Physiology, 157*(3), 615.

L'Heureux, N. (2003). *Tissue Engineered Blood Vessels and Methods and Apparatus for Their Manufacture - Patent 6503273.* Retrieved 10/4/2010, 2010, from http://www.freepatentsonline.com/6503273.html

L'Heureux, N., Paquet, S., Labbe, R., Germain, L., & Auger, F. A. (1998). A Completely Biological Tissue-Engineered Human Blood Vessel. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 12*(1), 47-56.

Liu, S. Q. (1998). Influence of Tensile Strain on Smooth Muscle Cell Orientation in Rat Blood Vessels. *Journal of Biomechanical Engineering, 120*(3), 313-320.

Mauck, R. L., Baker, B. M., Nerurkar, N. L., Burdick, J. A., Li, W., Tuan, R. S., & Elliott, D. M. (2009). Engineering on the Straight and Narrow: The Mechanics of Nanofibrous Assemblies for Fiber-Reinforced Tissue Regneration. *Tissue Engineering, Part B., 15*(2), 171.

Mauck, R. L., Soltz, M. A., & Wang, C. C. B. (2000). Functional Tissue Engineering of Articular Cartilage Through Dynamic Loading of Chondrocyte-Seeded Agarose Gels. *Journal of Biomechanical Engineering, 122*(3), 10/5/10-252-261. doi:10.1115/1.429656

Mitchell, S., & Niklason, L. (2003). Requirements for Growing Tissue-Engineered Vascular Grafts. *Cardiovascular Pathology, 12*(2), 59-64. doi:10.1016/S1054-8807(02)00183-7

Neidlinger-Wilke, C., Stalla, I., Claes, L., Brand, R., Hoellen, I., Rubenacker, S., Kinzl, L. (1995). Human Osteoblasts from Younger Normal and Osteoporotic Donors Show Differences in Proliferation and TGF-Beta Release in Response to Cyclic Strain. *Journal of Biomechanics, 28*(12), 1411.

Niklason, L. E., Gao, J., Abbott, W. M., Hirschi, K. K., Houser, S., Marini, R., & Langer, R. (1999). Functional Arteries Grown In-Vitro. *Science, 284*(5413), 489-493. doi:10.1126/science.284.5413.489

Niklason, L. E., Yeh, A. T., Calle, E. A., Bai, Y., Valentin, A., & Humphrey, J. D. (2009). Enabling Tools for Engineering Collagenous Tissues Integrating Bioreactors, Intravital Imaging, and Biomechanical Modeling. *Proceedings of the National Academy of Sciences,* doi:10.1073/pnas.0907813106

O'Donnell, T. F., Mackey, W., McCullough, J., Maxwell, S., Farber, S., Deterling, R., & Callow, A. (1984). Correlation of Operative Findings with Angiographic and Noninvasive Hemodynamic Factors Associated with Failure of Polytetrafluoroethylene Grafts. *1*(1), 12/10/2010, 136-148.

Pfister, B. J., Iwata, A., Taylor, A. G., Wolf, J. A., Meaney, D. F., & Smith, D. H. (2006). Development of Transplantable Nervous Tissue Constructs Comprised of Stretch-Grown Axons. *Journal of Neuroscience Methods, 153*(1), 95-103. doi:10.1016/j.jneumeth.2005.10.012

Rubbens, M. P., Mol, A., van Marion, M. H., Hanemaaijer, R., Bank, R. A., Baaijens, F. P., & Bouten, C. V. (2009). Straining Mode-Dependent Collagen Remodeling in Engineered Cardiovascular Tissue. *Tissue Engineering Part A, 15*(4), 841.

Sahoo, S., Cho-Hong, J. G., & Siew-Lok, T. (2007). Development of Hybrid Polymer Scaffolds for Potential Applications in Ligament and Tendon Tissue Engineering. *Biomedical Materials, 2*(3), 169-173. doi:10.1088/1748-6041/2/3/001

Schutte, S. C. (2010). Cyclic Strain Improves Strength and Function of a Collagen-Based Tissue-Engineered Vascular Media. *Tissue Engineering, Part A, 16*(10), 3149.

Seliktar, D., Black, R. A., Vito, R. P., & Nerem, R. M. (2000). Dynamic Mechanical Conditioning of Collagen-Gel Blood Vessel Constructs Induces Remodeling In-Vitro. *Annals of Biomedical Engineering, 28*(4), 351-362. doi:10.1114/1.275

Smith, D. H., Wolf, J. A., & Meaney, D. F. (2001). A New Strategy to Produce Sustained Growth of Central Nervous System Axons: Continuous Mechanical Tension. *Tissue Engineering, 7*(2), 131-139. doi:10.1089/107632701300062714

Sudhir, K., Hashimura, K., Bobik, A., Dilley, R. J., Jennings, G. L., & Little, P. J. (2001). Mechanical Strain Stimulates a Mitogenic Response in Coronary Vascular Smooth Muscle Cells via Release of Basic Fibroblast Growth Factor[*1]. *American Journal of Hypertension, 14*(11), 1128.

Syedain, Z. H., & Tranquillo, R. T. (2009). Controlled Cyclic Stretch Bioreactor for Tissue-Engineered Heart Valves. *Biomaterials, 30*(25), 4078-4084. doi:10.1016/j.biomaterials.2009.04.027

The Top 10 Causes of Death. (2008). Retrieved 3/1, 2010, from http://www.who.int/mediacentre/factsheets/fs310/en/index.html

Tower, T. T., Neidert, M. R., & Tranquillo, R. T. (2002). Fiber Alignment Imaging During Mechanical Testing of Soft Tissues. *Annals of Biomedical Engineering, 30*(10), 1221-1233.

Wilson, E., Mai, Q., Sudhir, K., Weiss, R. H., & Ives, H. E. (1993). Mechanical Strain Induces Growth of Vascular Smooth Muscle Cells via Autocrine Action of PDGF. *The Journal of Cell Biology, 123*(3), 741-747.

Zacharias, R. K., Kirkman, T. R., & Clowes, A. W. (1987). Mechanisms of Healing in Synthetic Grafts. *Journal of Vascular Surgery, 6*(5), 429-436.

Appendix A – Pairwise Comparison Chart (PCC)

Bioreactors	Accurate (10 ±1%	Adjustable size of ring/tube	High Throughput	Programmable	TEBVs easily secured	TEBVs easily removed	Media supply	Waste removal	Inexpensive	Totals	Percent Total
Accurate (10 ±1%		1	1	0.5	1	1	0	0.5	1	6	17.65 %
Adjustable size of ring/tube	0		0	0	0	0	0	0	1	1	2.94%
High Throughput	0	1		1	0.5	0.5	0	0	1	4	11.76 %
Programmable	0.5	1	0		1	1	0	0.5	1	4.5	13.24 %
TEBVs easily secured	0	1	0.5	0		0.5	0	0	1	3	8.82%
TEBVs easily removed	0	1	0.5	0	0.5		0	0	1	3	8.82%
Media supply	1	1	1	1	1	1		1	1	7	20.59 %
Waste removal	0.5	1	1	0.5	1	1	0		1	5.5	16.18 %
Inexpensive	0	0	0	0	0	0	0	0		0	0.00%

Appendix B – A Guide to Heat Shrink Tubing

Goals:

- Effectively seal silicone tubing to 1/16” inner diameter male barbed bayonet luer fittings

Materials:

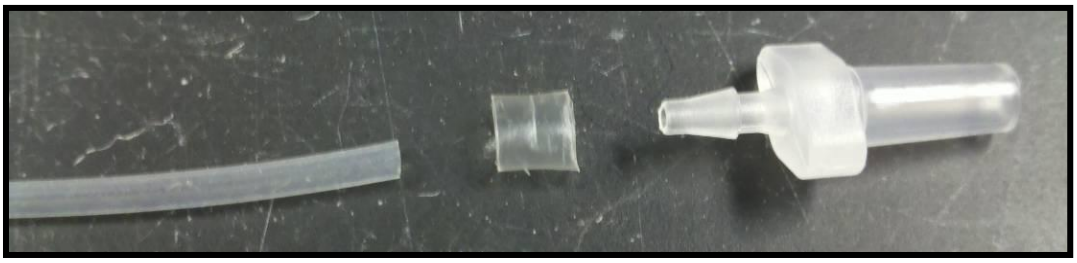
- Scissors/scalpel
- Heat gun (preferably with low and high heat settings)
- Tweezers/forceps
- Gloves
- Biocompatible heat shrinkable tube (1/8" diameter)
- Silicone tubing
- Male barbed bayonet luer fitting for 1/16" inner diameter tubing

Procedure:

1: Using gloves to handle the silicone tubing, cut the tubing to desired length (10cm)

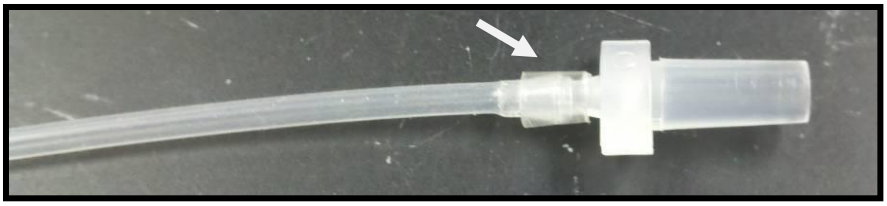
Note: For more accurate, clean cuts, use a scalpel on a flat surface alongside of a measuring device

2: Cut heat shrink tubing to desired length (3-5mm or long enough to cover the barb and silicone tubing on the luer fitting) (see Figure below for approximate scaled sizing)



3: Using gloves to handle the silicone tubing, attach the silicone tubing to the barbed end of the luer fitting, ensuring that the tubing is secured above the barb (see Figure below)

4: Using gloves, cover the silicone tubing and barbed tip overlap with heat shrink tubing (see Figure below)



5: Remove your gloves (in case of heat exposure) and use tweezers/forceps to pick up the luer fitting by its' hollow end (see Figure below)

6: Position the heat gun pointing away from yourself and turn it on (depending on the type of heat gun used, this may require an additional person to hold the heat gun while the original person completes steps 7-9) (see Figure below for model heat gun we used)

7: Hoist the silicone tubing—heat shrink tubing—luer fitting combination approximately 1.5 inches from the mouth of the heat gun using the tweezers/forceps and spin continuously for 10-30 seconds to adhere the heat shrink tubing to all sides of the luer fitting (*BEWARE of melting the luer fitting due to positioning the parts too closely to the heat gun) (see Figure below)

8: Repeat for multiple tubing assemblies

9: Switch the heat gun to the cool setting for a minimum of one minute before shutting the heat gun off and storing it

Appendix C – A Guide to Wiring and Sealing Motors

Potting and Sealing Motors

Materials:

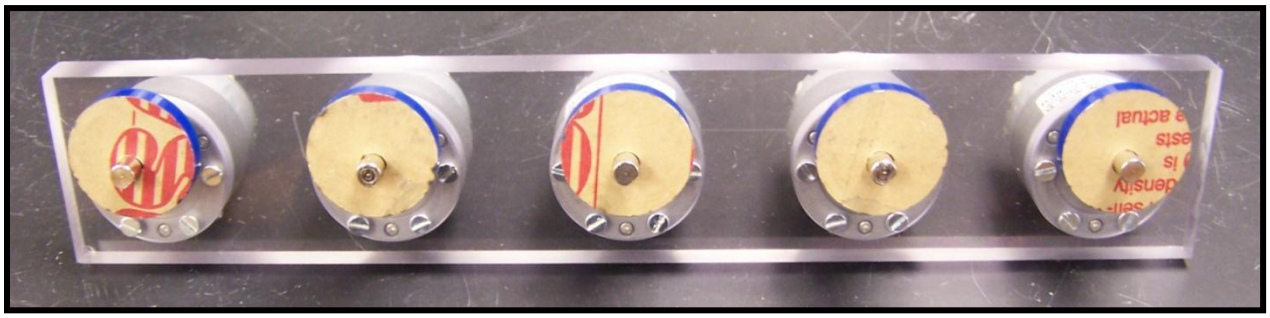
- 12 V 60 RPM DC motor (x5) mounted into motor mount
- Epoxy, hysol M-121hp medical device epoxy adhesive (50 mL)
- Hysol adhesive for encasing, urethane, (50 mL)
- Potting gun
- Easy-ID low voltage cable, 24/2 AWG, 0.11” W x 0.06” thick, 12 VDC, 50’ L
- Wire cutters
- Heat gun
- Soldering kit
- Vice
- Moisture-seal polyolefin heat-shrink tubing, 1.5” ID before, 0.75” ID after – cut into 2.5” lengths (x5)
- Small diameter heat shrink tubing for wiring

Procedure (Note – do everything listed for each motor individually):

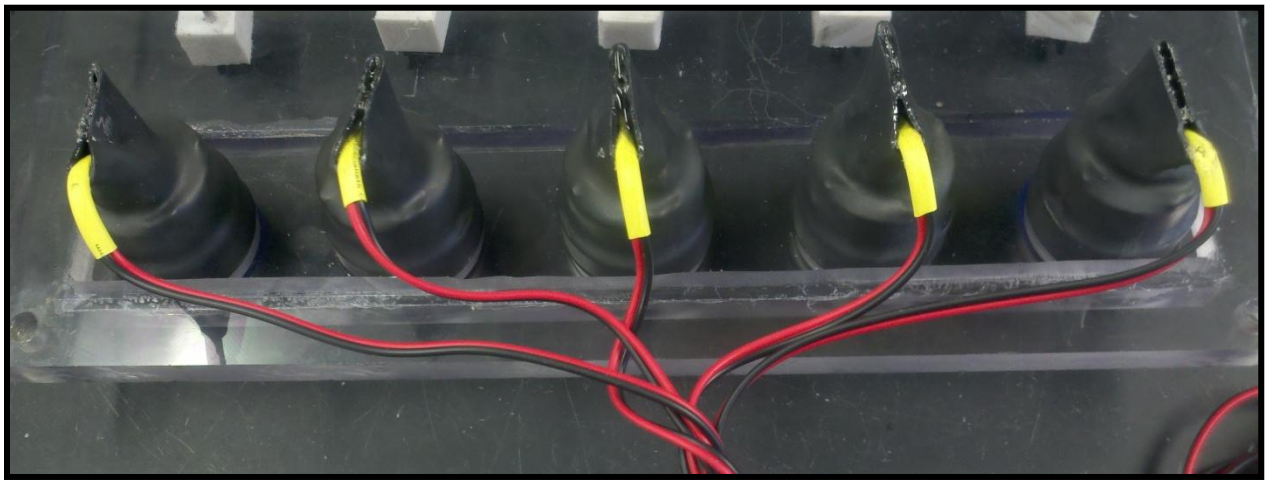
1. Cut wire to 6 foot lengths
2. Strip wire and fix to motor leads
3. Solder wire to motor leads
4. Heat shrink positive and negative wires near soldering point to prevent further separation of zip wire
5. Secure motor mount into vice facing upside down with wires coming out the top
6. Pot motor holes with epoxy hysol M-121 medical device epoxy and potting gun
7. Once all motors have been potted, flip motor mount in vice so epoxy drips down wires (and not into motor)
8. Let dry for 6 hours
9. Attach moisture-seal heat-shrink tubing to outside of motors (encasing both the gearbox and motor)
10. Use heat gun to permanently attach tubing around motor
11. Fill remaining area within heat shrink tubing with hysol adhesive for encasing
12. Position wires in the same direction perpendicular to the long side of the motor mount and pinch the heat shrink tubing and hysol adhesive encasing closed around the wire

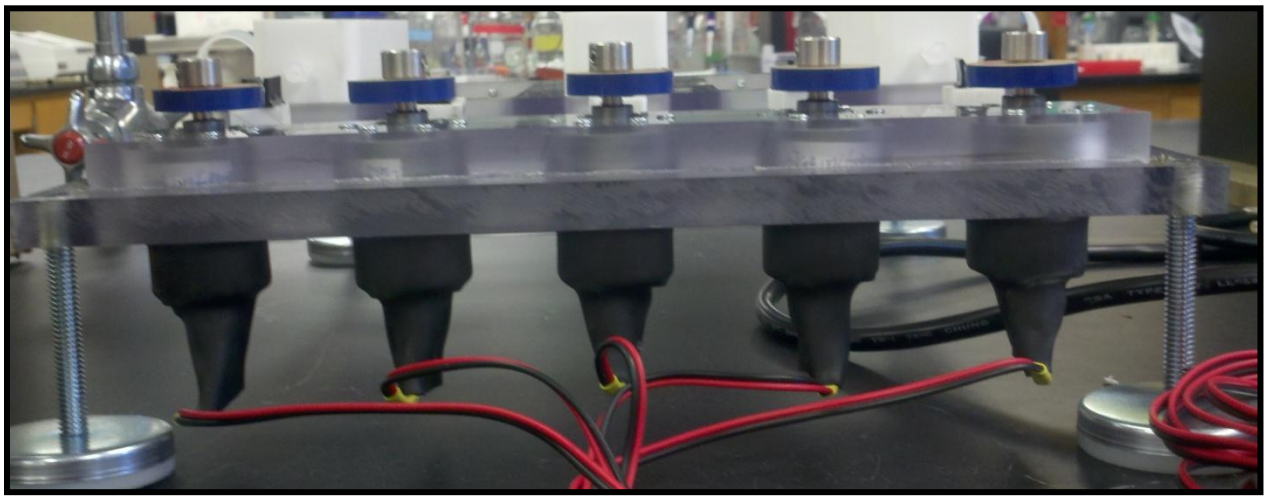
13. Let dry for 24 hours

Before:



After:





Wiring Motors to Power Supply

Materials:

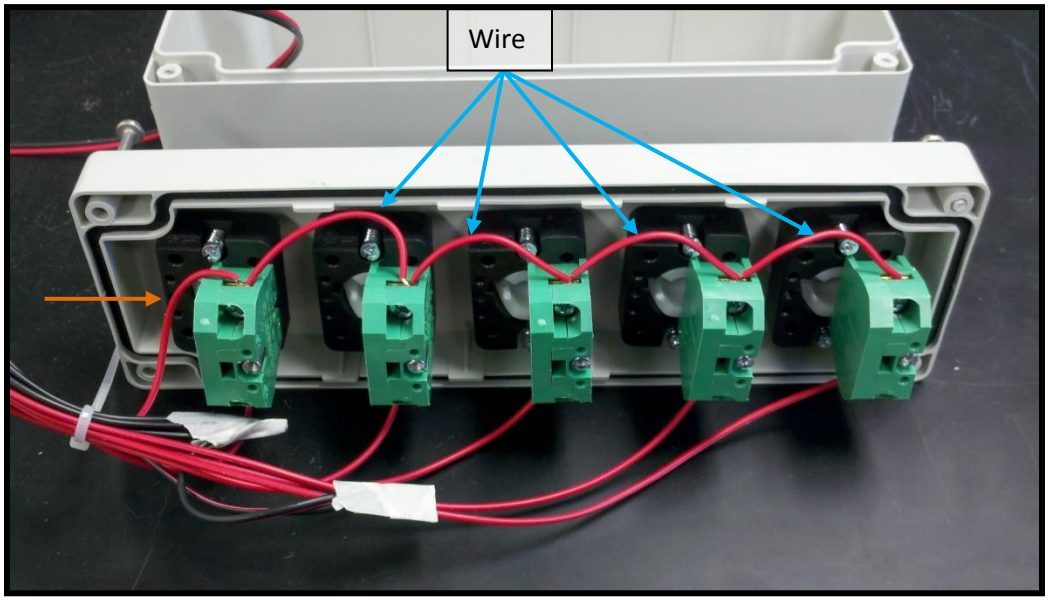
- Motors sealed into motor mount and base
- 5-hole 22mm button enclosure, plastic
- Selector switch, 22mm, plastic (x5) + contact blocks
- Easy-ID low voltage cable, 24/2 AWG, 0.11” W x 0.06” thick, 12 VDC, 50’ L
- Wire cutters
- Wire connectors (male and female pins) (x10)
- 16 AWG power cable

Procedure:

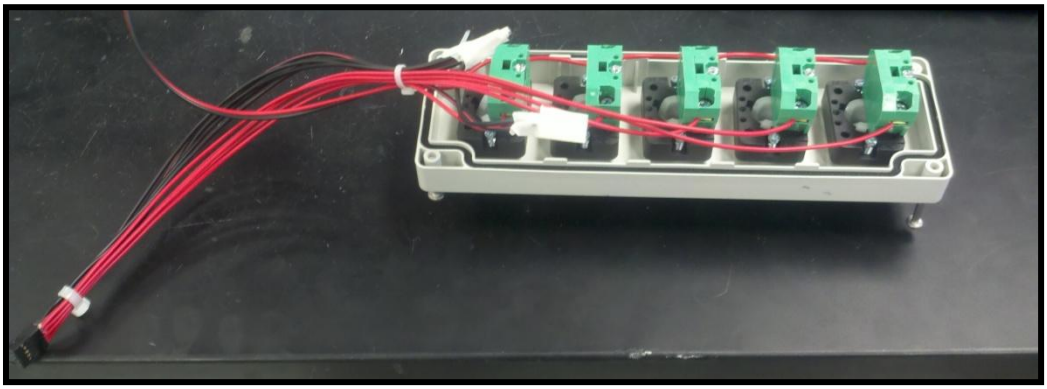
1. Assemble the button enclosure as per instructions given with purchase

2. Measure, cut, and strip (all wires should look like this when stripped) the ends of four 5 cm long pieces of positive (red) 24/2 AWG 12 VDC wire (see Figure below)

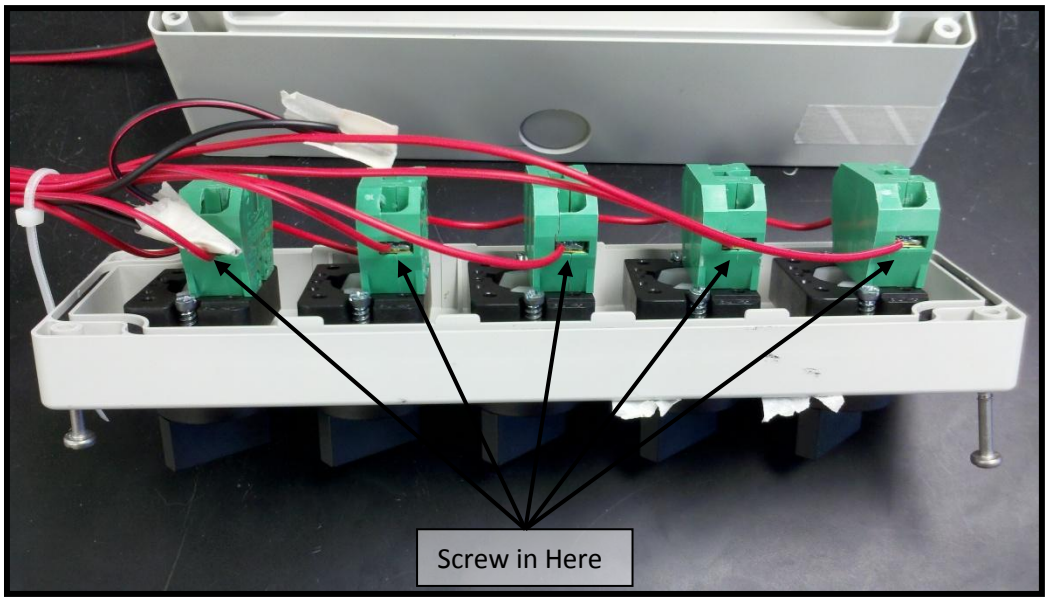
3. Attach and screw the pieces of wire between the 5 selector switches (see blue arrows in Figure below)



4. Measure, cut, and strip the ends of five pieces of positive (red) 24/2 AWG 12 VDC wire so all wires feed out of the same side of the 5-hole 22mm button enclosure and end at approximately the same point (see Figure below)



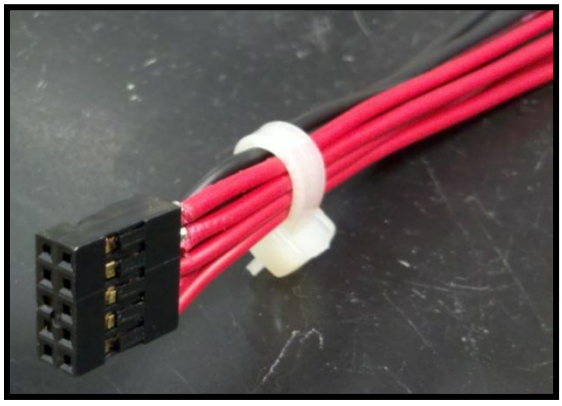
5. Attach and screw one length of wire to each contact block (see Figure below)



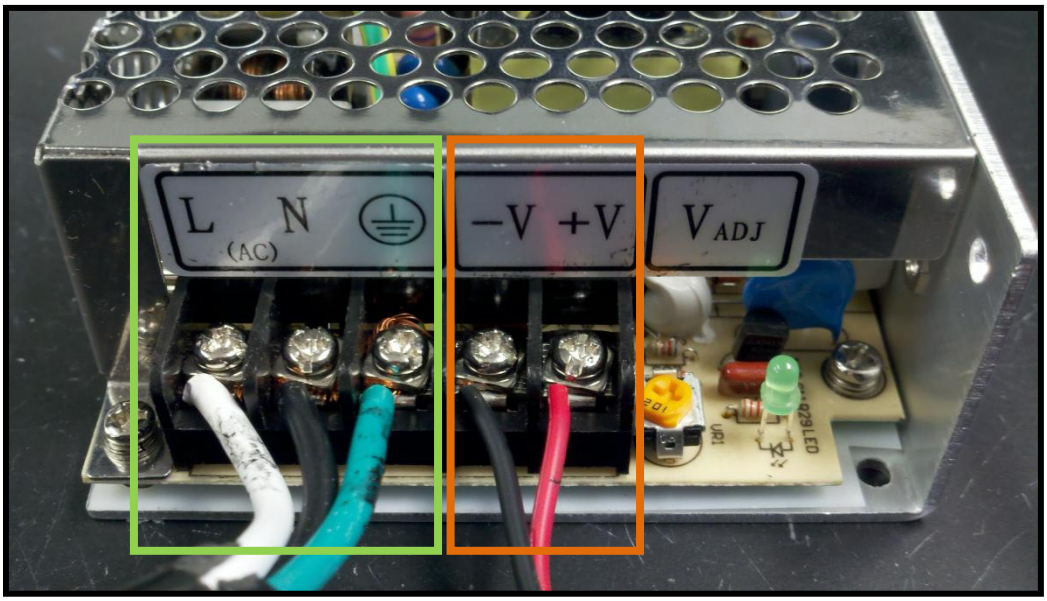
6. Strip the other end of the same lengths of wire and crimp push-in wire connector pieces to the ends of each wire and fit into plastic alignment case, ensuring wires of the same color are all secured on the same side

7. Cut and strip 5 lengths of negative (black) 24/2 AWG 12 VDC wire to equal lengths

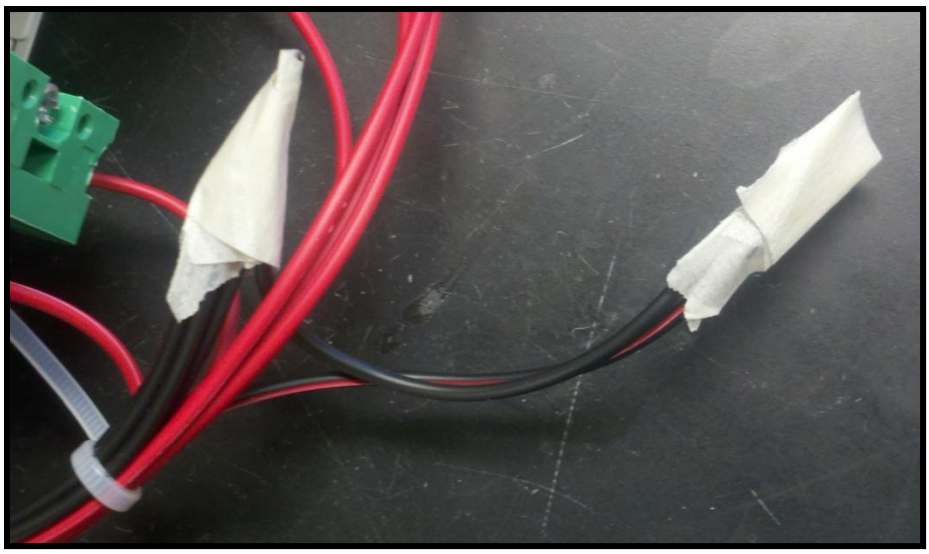
8. Crimp push-in wire connector pieces to the ends of these lengths of wire and fit into the same plastic alignment case, ensuring wires of the same color are all secured on the same side (see Figure below)



9. Cut and strip a 60 cm length of wire (still zip connected together) and attach one end of the negative (black) wire and positive (red) wire to the power supply and secure with a screw (see orange box in Figure below)



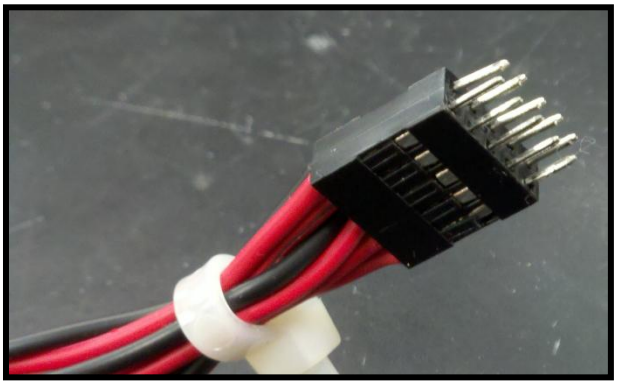
10. Cut and strip a 5 cm long piece of negative (black) wire and twist together with all other free ends of negative (black) wires, securing together with tape (see Figure below)



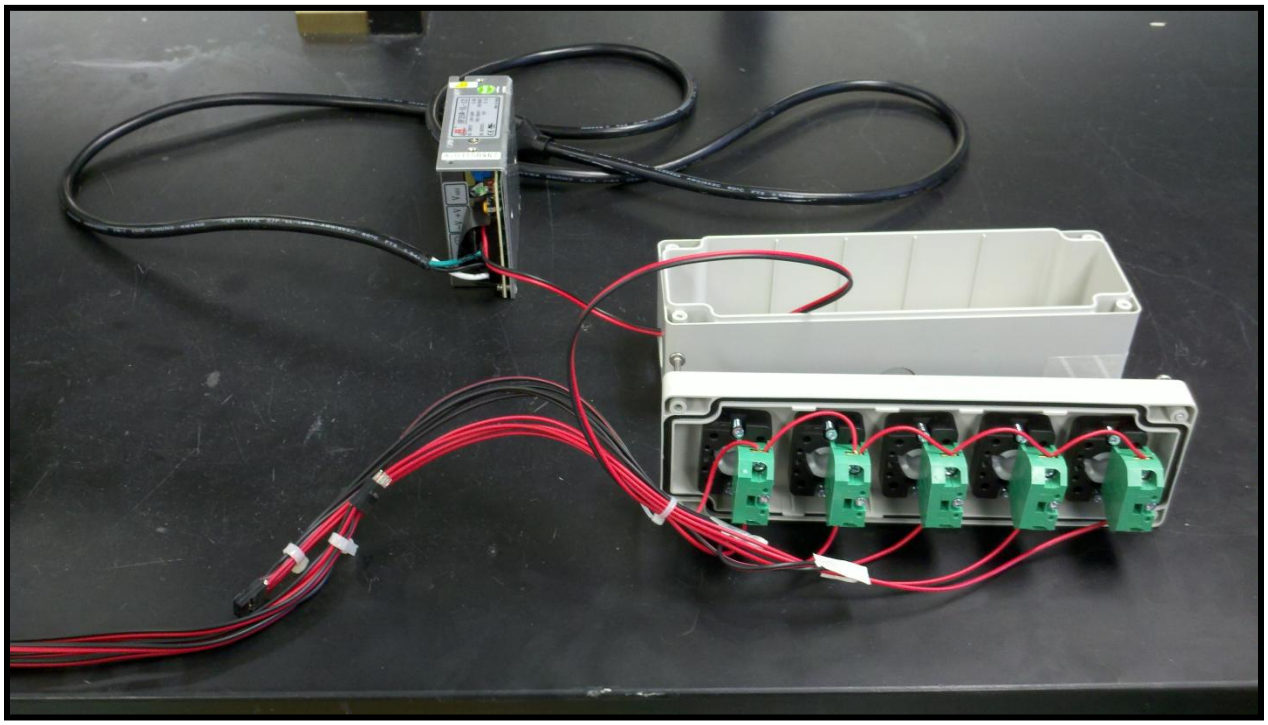
11. Attach the free end of the positive (red) wire to the contact block nearest the 22 mm button enclosure's exit hole (see orange arrow in the Figure in step 3)

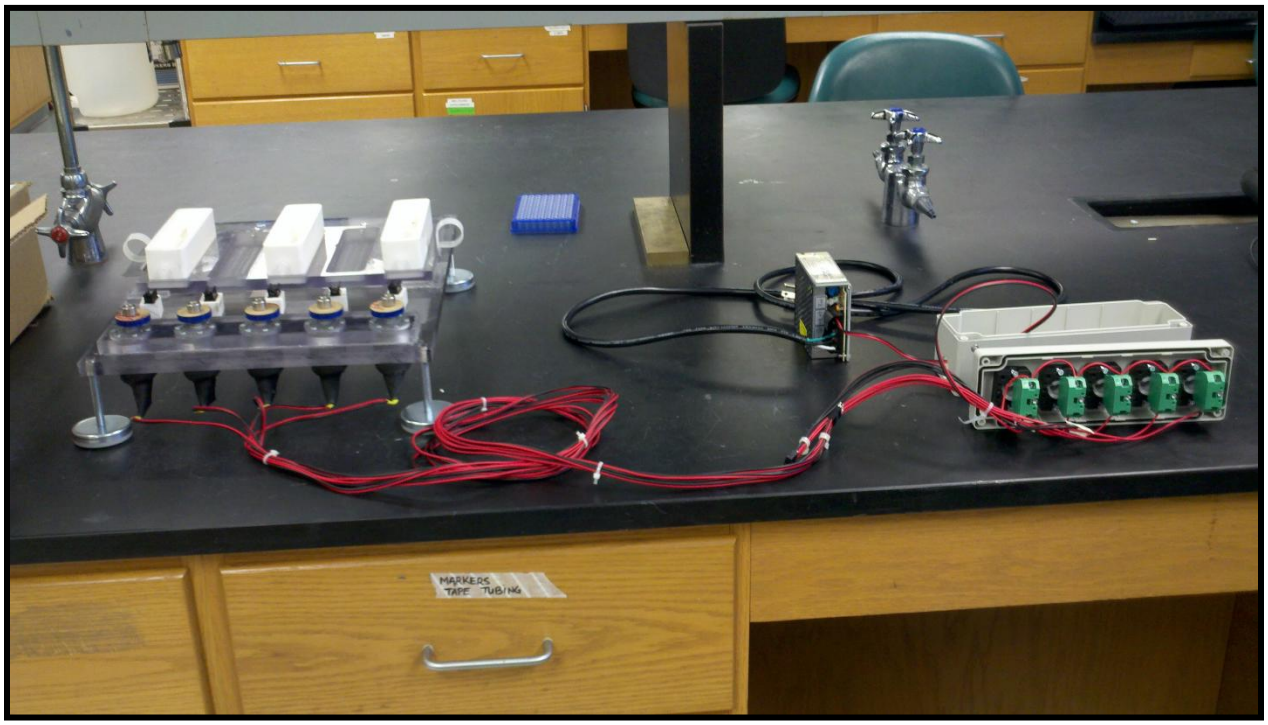
12. Attach the 16 AWG power cable to the power supply (see green box in Figure in step 9) and cover all wires with electrical tape

13. Strip free ends of 6 foot long wire sealed into 60RPM 12V DC motors and crimp push-in wire connectors onto lengths of wire and fit into plastic alignment case, ensuring wires of the same color are all secured on the same side (see Figure below)



Final Product:

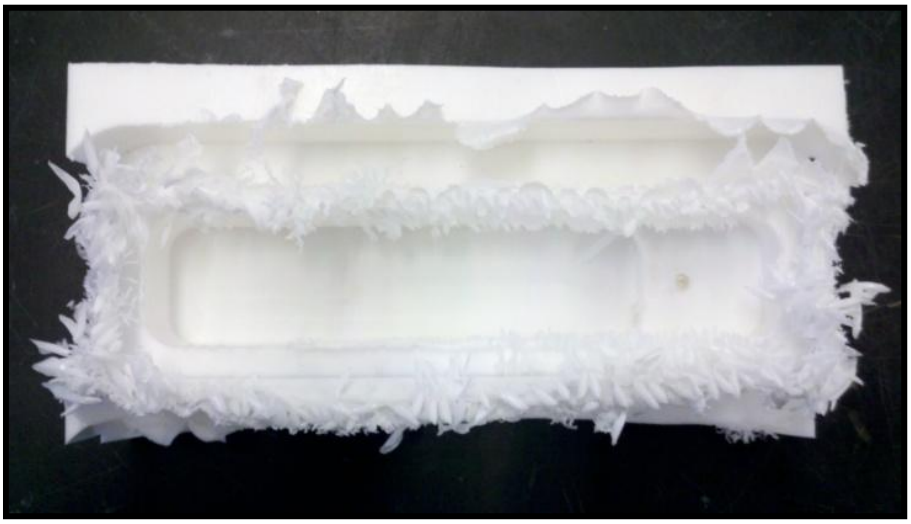




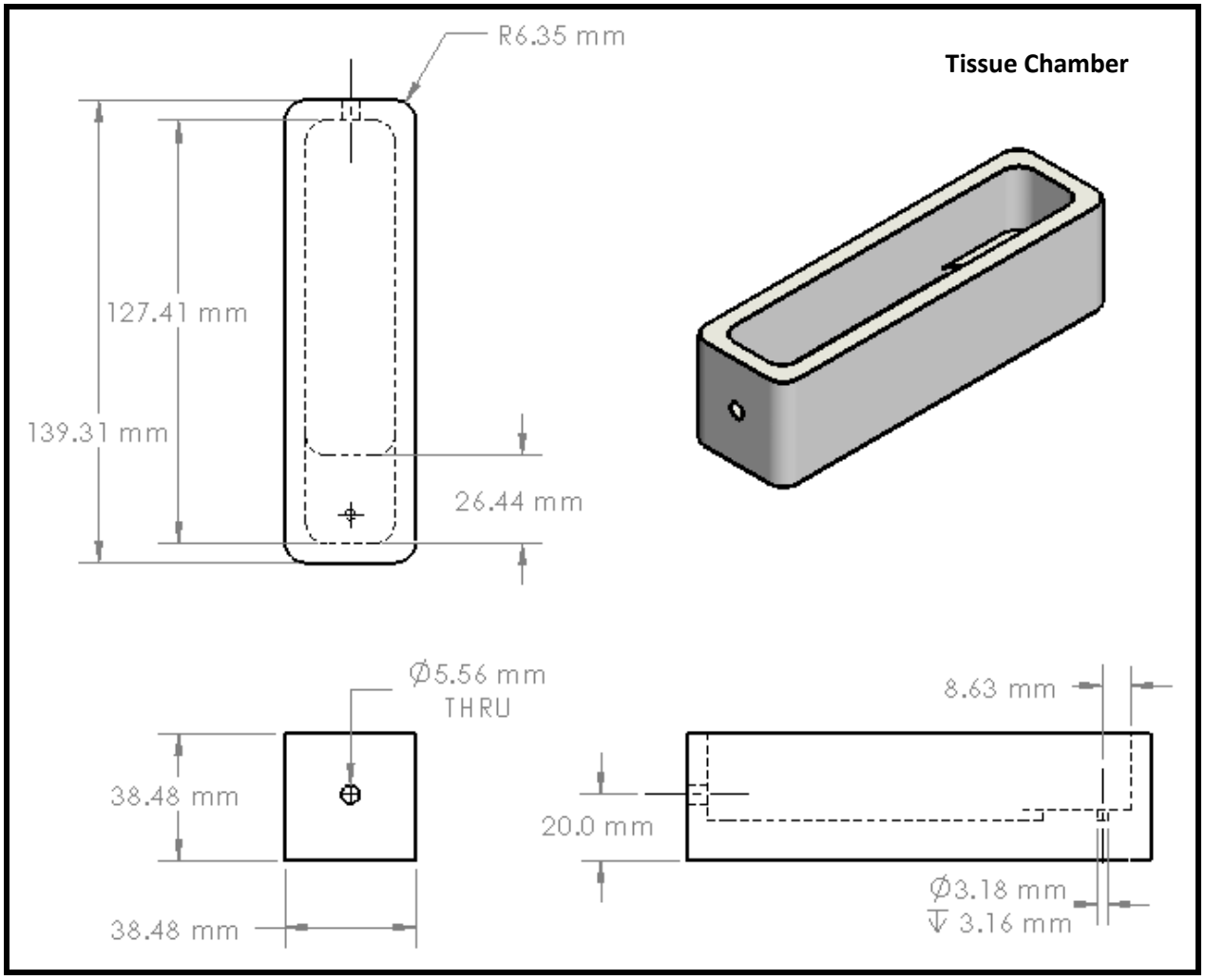
Appendix D – Building Our Device

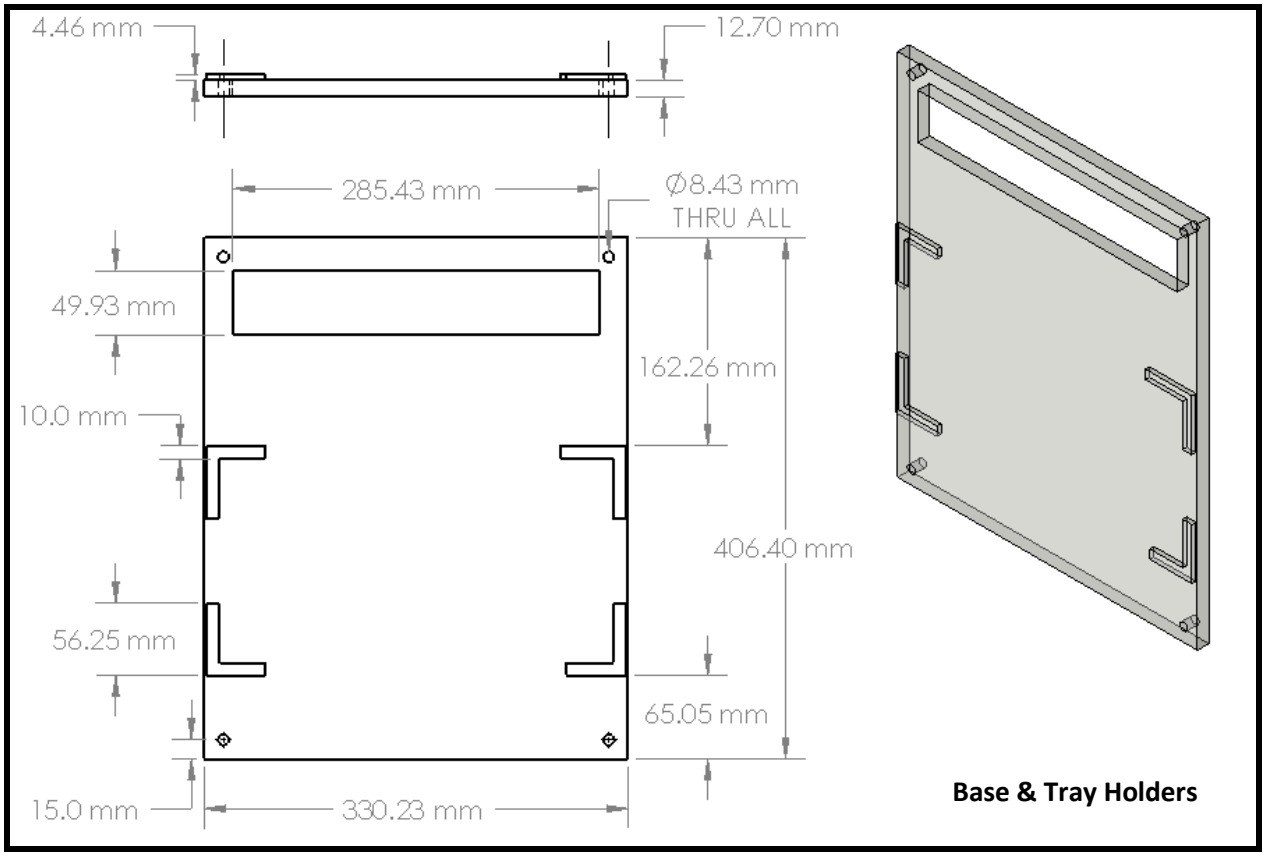
The following engineering drawings profile the geometry and dimensions of the components of the device that were machined. These include the tissue chamber, base, motor mount, and removable tray. All of these components were manufactured from raw plastic sheets using high-speed milling techniques in a HAAS automated mill. The following models and dimensions were programmed using SolidWorks and FeatureCAM softwares, and were uploaded into the mill's computer system.

HAAS Milling Machine

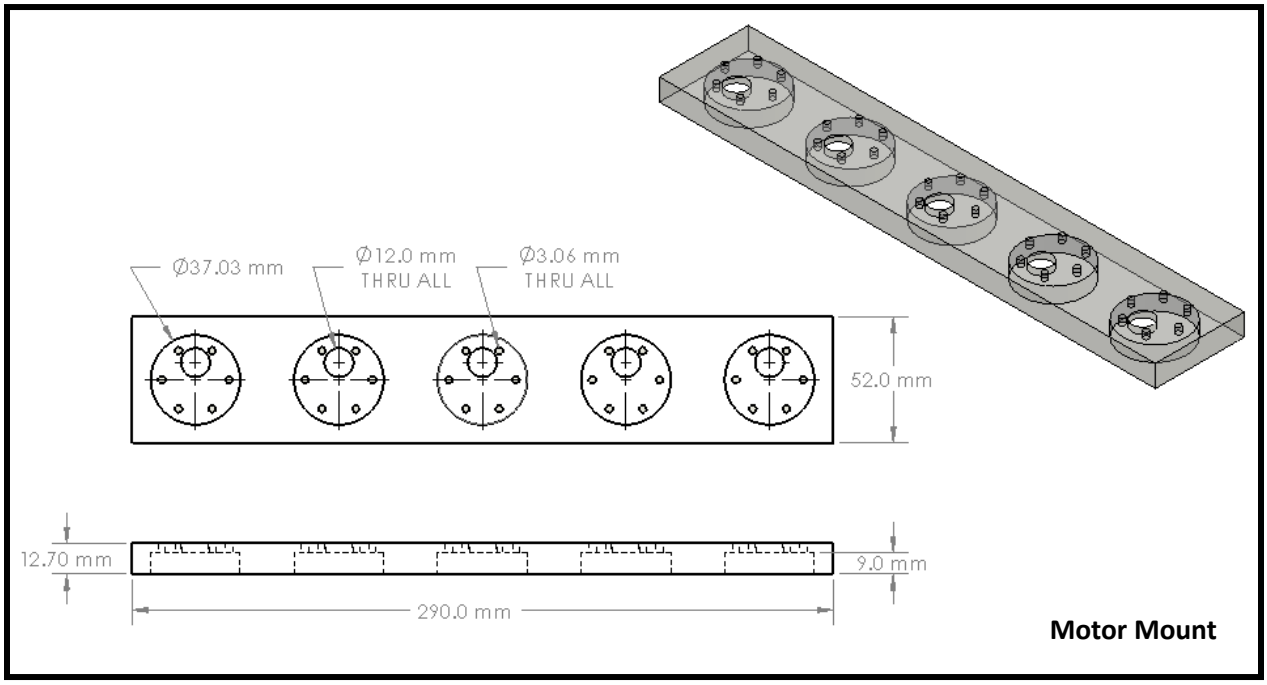


Partially Milled Tissue Chamber

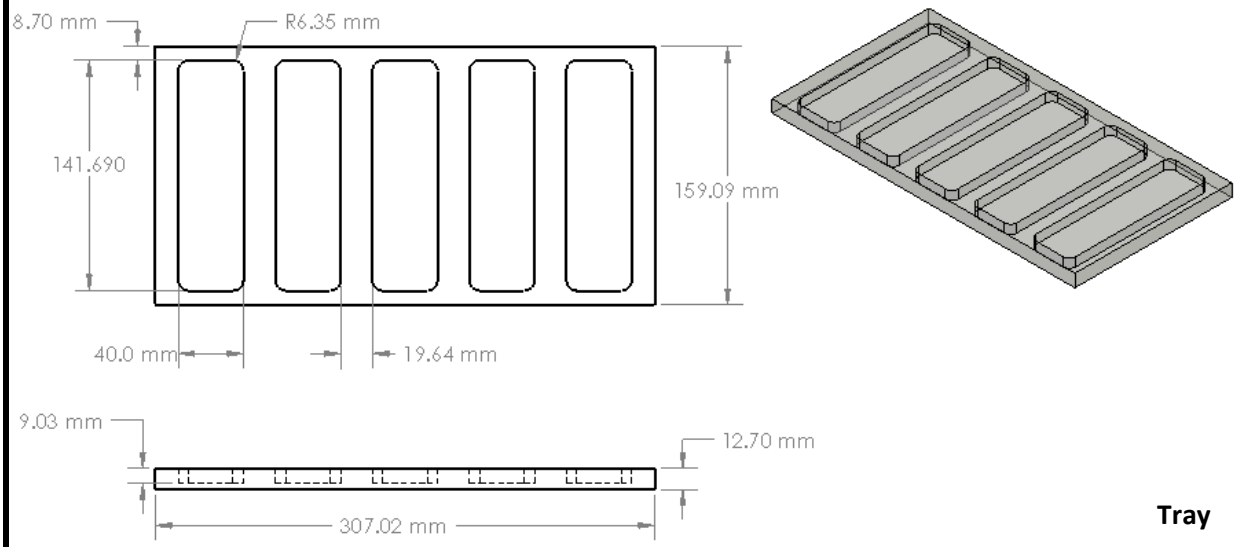

Tissue Chamber
R6.35 mm
127.41 mm
139.31 mm
26.44 mm
Ø5.56 mm
THRU
38.48 mm
38.48 mm
8.63 mm
20.0 mm
Ø3.18 mm
↧ 3.16 mm



Base & Tray Holders



Motor Mount


8.70 mm
R6.35 mm
141.690
159.09 mm
40.0 mm
19.64 mm
9.03 mm
12.70 mm
307.02 mm

Tray

Appendix E – User Manual

Prepare autoclaved tools & materials:

- Forceps (narrow tip, 45^0 and/or 90^0)
- Forceps (blunt curved tip)
- Flat spatula (for grease)
- Aspirator tips
- 10 cm silicone tube heat shrunk to 1/16" inner diameter female barbed polypropylene luer (x5)
- Tissue chamber (x5) with stainless steel dowel pin fitted into pinch clamp hole and polypropylene female ¼-28 threaded luer mounted into chamber wall
- Tissue chamber lids (x5)
- Compression spring (number dependent on number of mechanically conditioned tissue chambers in use)
- Silicone-based high vacuum grease (Dow Corning, Midland, MI) (as needed)

Non-autoclaved, sterile materials:

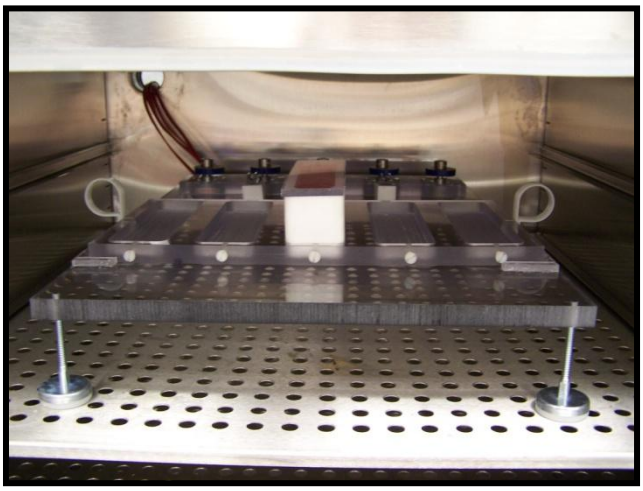
- Latex gloves (as needed)
- 10 & 25 mL setological pipets (as needed)
- Sterile Monoject® 1mL TB syringe (x5)
- 25 cm^2 tissue culture flask (x2)
- 90mm (or larger if preferred) tissue culture dish (x2)
- 140mm tissue culture dish
- Culture media (DMEM, 1X with 4.5 g/L glucose, L-glutamine, & sodium pyruvate) (~50 mL)
- Complete culture media (DMEM, 1X with 4.5 g/L glucose, L-glutamine, & sodium pyruvate – modified with 10% FBS, 1% Penicillin/Streptomycin) (~300 mL)

Tools & materials cleaned with 70% ethanol solution:

- Pipetter
- Aspiration hose
- Base of device (including motors+ cams + syringe holders)
- Tray of device

Incubator Set-up Procedure:

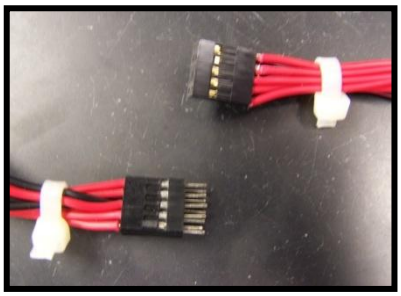
1.) Spray and wipe down the base component of the device and position in the incubator with the motors furthest from, and parallel to, the front of the incubator.

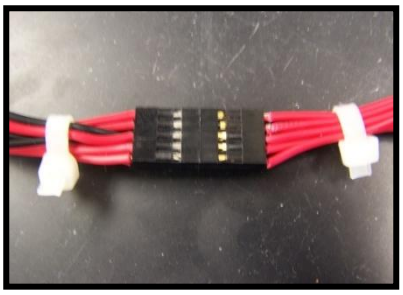


2.) Feed the wires through the hole at the back of the incubator.

3.) Arrange the switches and power supply on top of or next to the incubator near an electrical outlet and away from any liquids.

4.) Connect the male and female wire connectors (red wires to red wires; black wires to black wires), being very careful not to bend any of the pins in the process (if bent, motors may not turn on – can be fixed by straightening or pulling on pins).



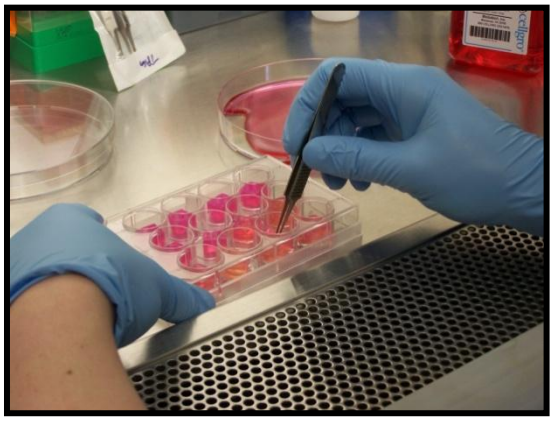


5.) Plug in the power supply.

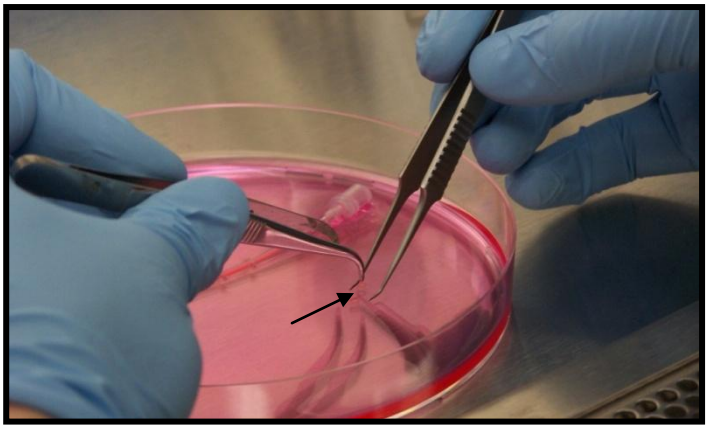
Ring Loading onto Silicone Tube Procedure:

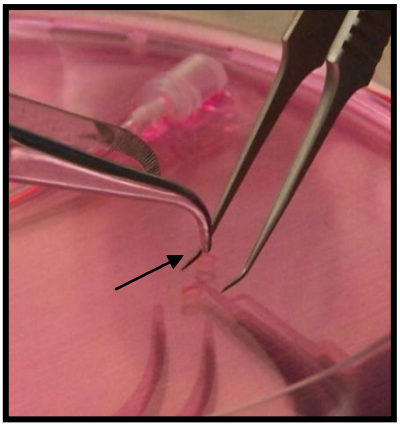
*Note – All procedural steps take place within a sterile biosafety cabinet unless otherwise specified. Whenever leaving the biosafety hood, be sure to apply 70% ethanol before re-entering to control contamination risk.

1.) Fill base of 140mm tissue culture dish with ~ 25mL culture media.
2.) Aspirate media out of tissue ring culture molds.
3.) Carefully remove tissue rings from culture molds using narrow 90^{o} forceps and submerge in tissue culture dish media.

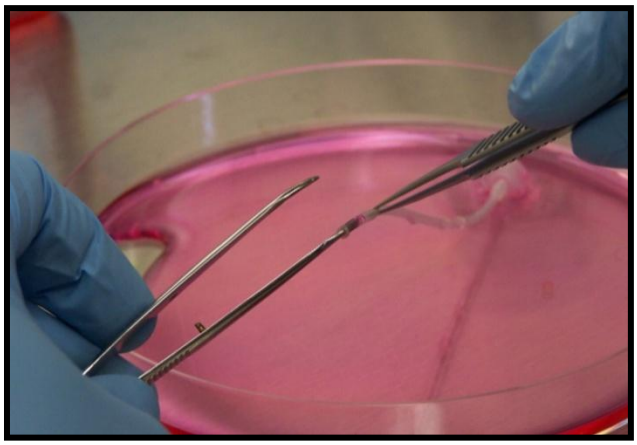


4.) Repeat for all tissue rings.
5.) Open autoclaved silicone tubing and place within tissue culture dish with tissue rings.
6.) Using narrow forceps, place tissue ring (arrow) around one open grip of the blunt curved forceps and angle blunt curved forceps upwards in media to prevent ring sliding.

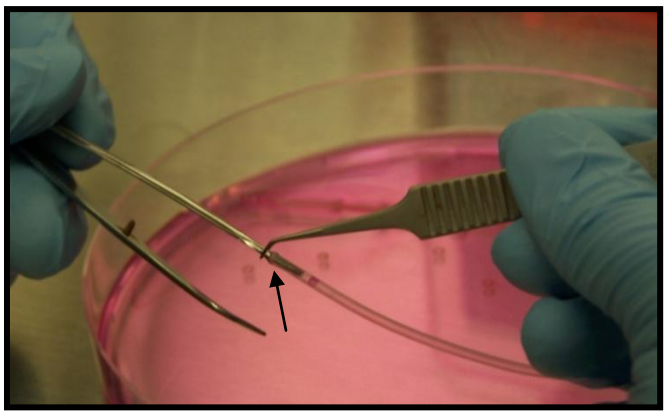


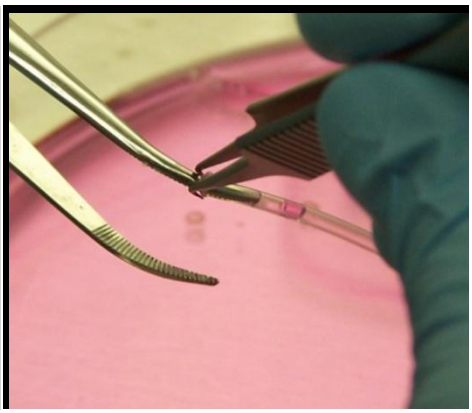


7.) Use narrow forceps to place the open end of the silicone tubing tightly over the open grip containing the tissue ring.

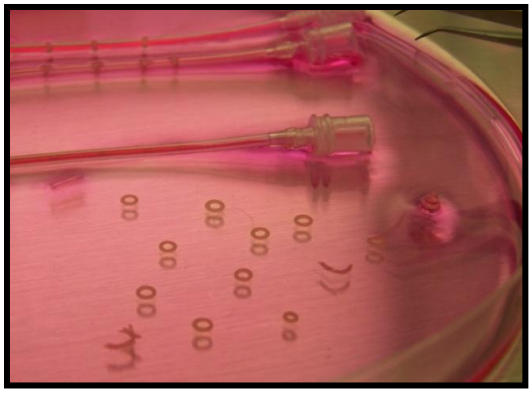


8.) Use narrow forceps to slide the tissue ring (arrow) onto the silicone tube and position it approximately 2 cm from the luer.

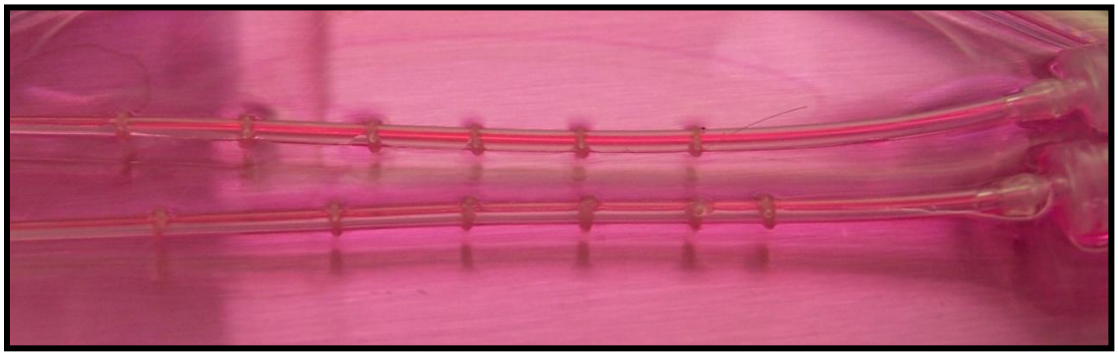




9.) Let silicone tube and tissue rings remain submerged in culture media while loading other rings and samples.



10.) Repeat steps 6-9 until 5-6 tissue rings have been successfully loaded ~1 cm apart on the silicone tube.

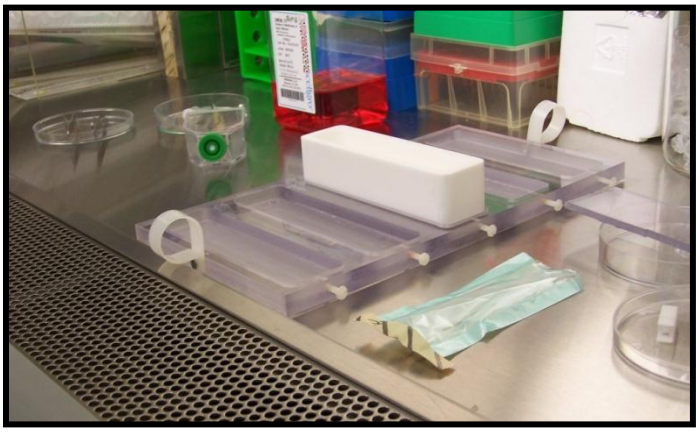


11.) Repeat steps 5-10 until 5 silicone tubes have been loaded with 5-6 tissue rings each (Note – if using less tissue chambers, use fewer silicone tubes, lids, and syringes.

Tissue Chamber Loading Procedure:

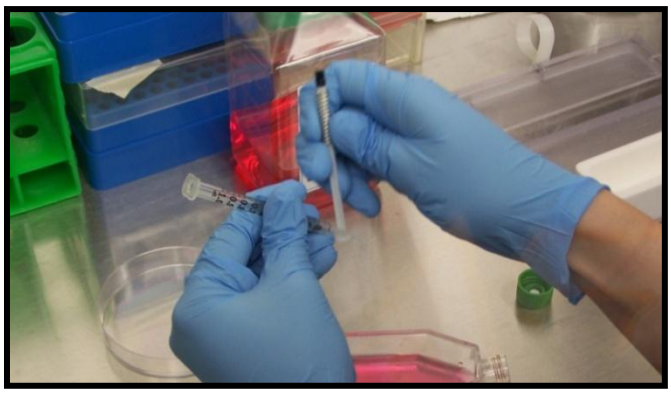
*Note – All procedural steps take place within a sterile biosafety cabinet unless otherwise specified. Whenever leaving the biosafety hood, be sure to apply 70% ethanol before re-entering to lessen contamination risk.

1.) Set up biosafety cabinet as seen in Figure # below, adding more tissue chambers if necessary.

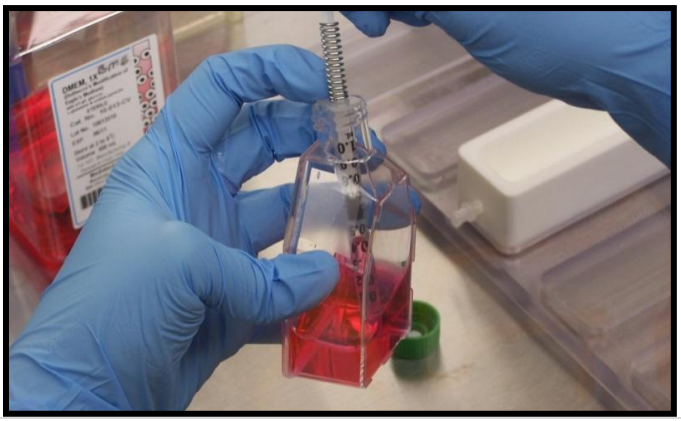


2.) Fill tissue culture flask with ~25 mL of culture medium using pipetter.

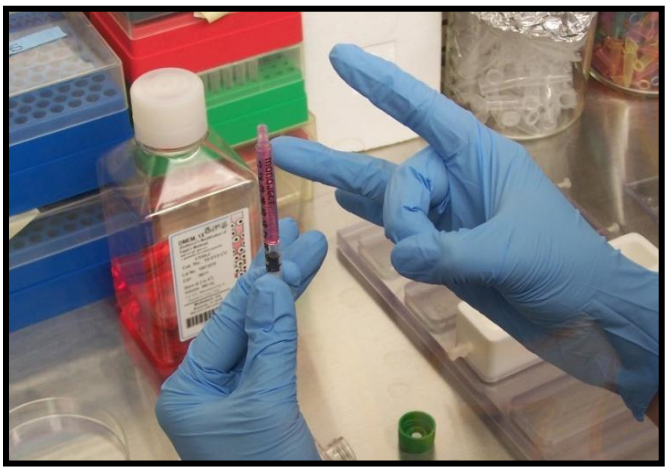
3.) Remove syringe plunger, place compression spring around plunger, and reinsert plunger into the syringe barrel.



4.) Depress syringe plunger until spring is fully compressed, insert syringe into tissue culture flask, and fill syringe to the 0.9 mL mark on the syringe barrel.



5.) Invert the syringe and tap out any extra air bubbles that may exist within the syringe barrel.

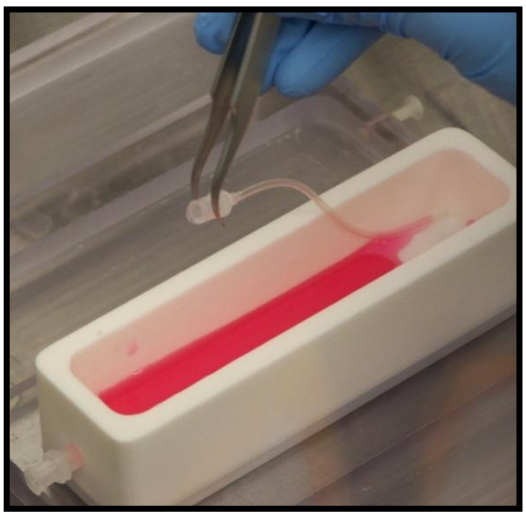


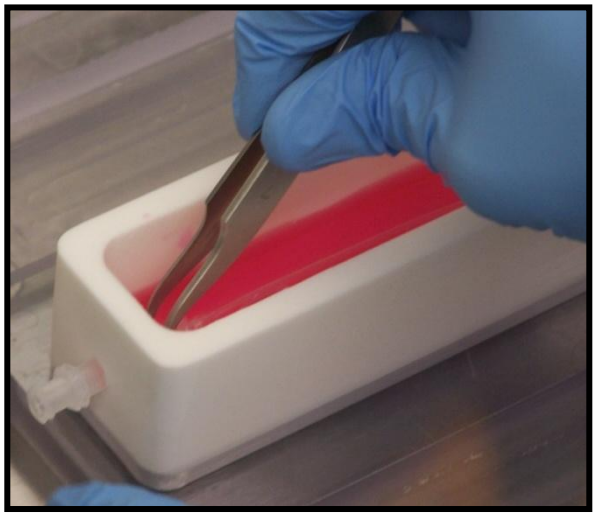
6.) Depress the plunger to remove the excess air that floats to the syringe tip during tapping and place the syringe in a sterile petri dish.

7.) Fill the tissue chamber with ~25 mL of complete culture medium (with FBS and P/S added).

8.) Using forceps, remove a tissue ring loaded silicone tube from the 140 mm culture dish and carefully place it into the tissue chamber.

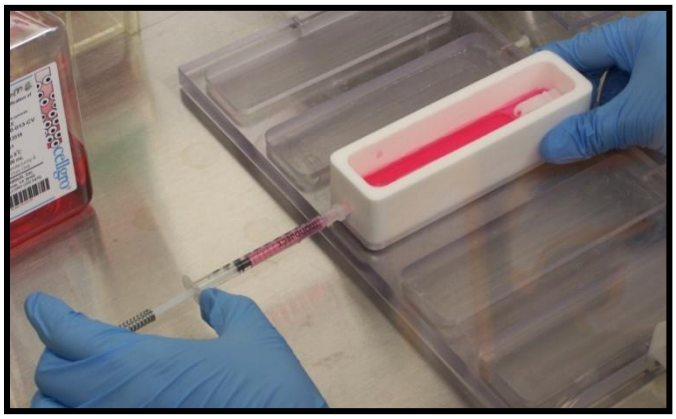
9.) Using foreceps, grip the luer attached to the silicone tube and fit in tightly over the luer threaded into the chamber wall.



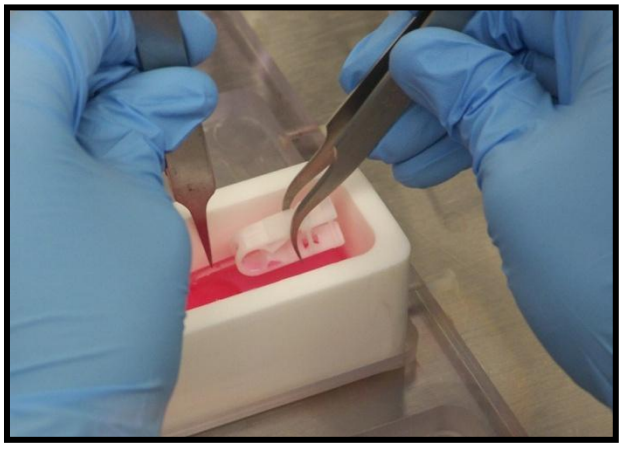


10.) Insert the 1 mL push-connect syringe into the outer opening of the threaded luer in the tissue chamber wall.

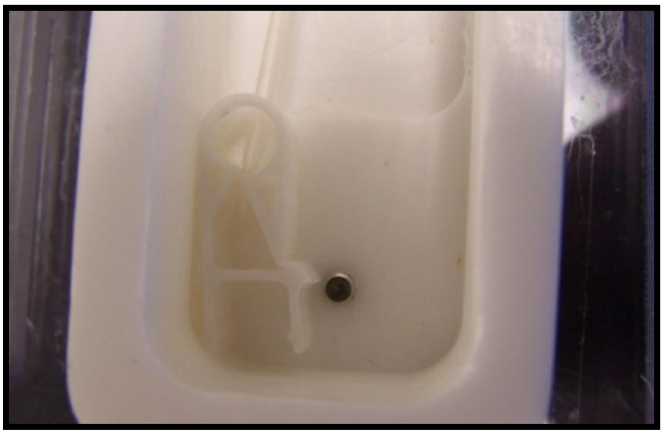
11.) Depress the syringe plunger until the compression spring is flush with the plunger head and syringe barrel.



12.) Use a pair of forceps to hold a pinch clamp in one hand, use the other hand to push the free end of the silicone tube through the pinch clamp opening.

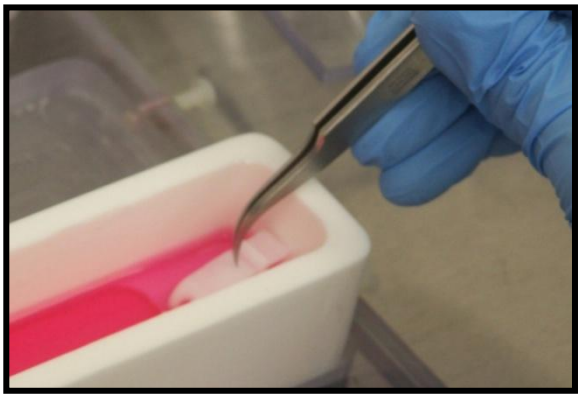


13.) Hold the pinch clamp sideways onto the base of the tissue chamber with a pair of forceps with one hand, and with your other dominant hand, grip the inside of the silicone tube using narrow tipped forceps and pull or slide the silicone tube beyond the clamping point. Be very careful not to damage the tubing or pull it more than half a centimeter past the clamping point.



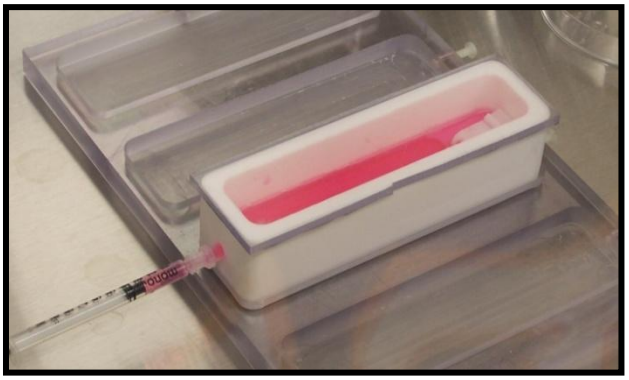
14.) Place the pinch clamp onto the steel dowel at the base of the tissue chamber.

15.) Use one hand to pressurize the syringe plunger down to 0.2 mL and the second hand to press the pinch clamp closed using a pair of forceps.



16.) Fill the tissue chamber with an additional ~35 mL of complete culture medium (with FBS and P/S added).

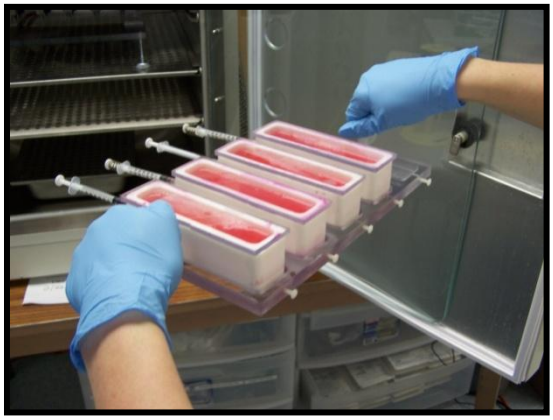
17.) Cover the tissue chamber with a sterile lid.



18.) Position the tissue chamber within the tray with the clamp-end nearest to the plastic screws and secure the tissue chamber into the base by hand-tightening the screw (can further tighten once removed from the biosafety cabinet).

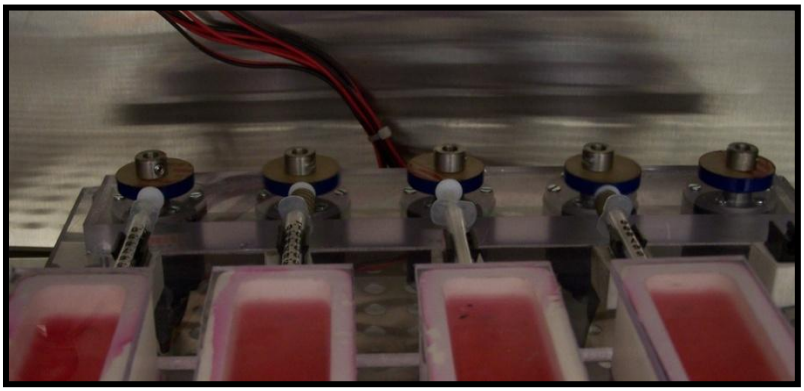
19.) Repeat steps 2-19 until all tissue chambers are secured and ready for transport from the biosafety cabinet to the incubator.

20.) Carefully lift the tray by its handles with the syringes pointing away from you and transport it to the incubator.

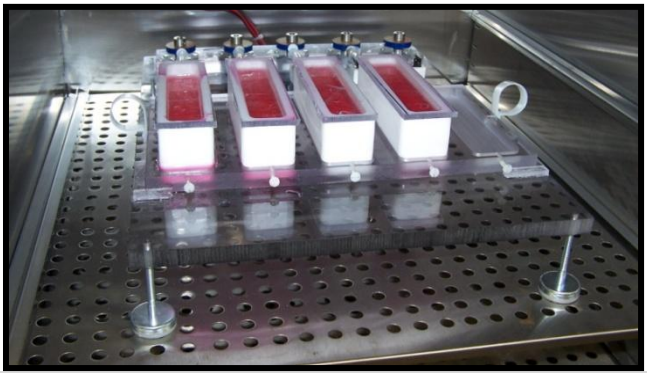


21.) Position the tray within the tray holder, with syringes on top of the syringe holders in the incubator.

22.) Depress the syringe plunger so that it's flush with the cam, and snap the syringe(s) into place.



23.) Close the incubator doors and switch on the mechanically conditioned tissue chambers.



Appendix F – Bill of Materials

Item Description	Item/Part Number	Distribution Co.	Quantity	Price	Total
303 Stainless Steel 6mm Bore Shaft Collar with M4 Set Screw	57485K23	McMaster-Carr	5	3.96	19.8
UHMWPE (12" length x 12" width x 1.5" thick) White Sheet	8270K81	McMaster-Carr	1	53.79	53.79
Abrasion Resistant PC (24"x12"x0.5") Sheet	8707K152	McMaster-Carr	1	67.6	74.68
Motor (12V, DC, 60 RPM, High Torque Gearbox)	001480-115	Virtual Village	5	8.99	45.81
25 Watt, 12V/2.1A Single Output Power Supply	PS1-25W-12	Circuit Specialists	1	14.9	23.35
Epoxy, Hysol M-121hp Medical Device Epoxy Adhesive (50 ml)	7370A37	McMaster-Carr	1	15.05	15.05
Hysol Adhesive for Encasing, Urethane, (50ml)	7369A33	McMaster-Carr	1	16.54	21.16
5-Hole 22mm button enclosure unit, plastic	SA111	Automation Direct	1	11.75	11.75
Moisture-seal polyolefin heat-shrink tubing, 1.5" ID before, 0.75" ID after, 48" L, Blac	74965K68	McMaster-Carr	1	24.44	24.44
316 SS dowel pin (1/8" diameter, 1/4" length) x10/pk	97395A439	McMaster-Carr	1	5.43	5.43
Multiline Plastic Clamp (1/4" outer diameter, 10 slots)	2216T13	McMaster-Carr	1	1.44	1.44
PP Level Feet (10-24 thread, 1-13/64" base D, 1.5" bolt length)	62805K31	McMaster-Carr	4	1.2	4.8
Female luer 1/4-28 thread mount to male luer, PP, 100/pk	FTLLBMLRL-6	Value Plastics	1		0
Female luer lug to barb, 1/16" ID tubing, PP, 100/pk	FTL10-6	Value Plastics	1		0
Selector switch, 22mm, plastic, black	GCX3300	Automation Direct	5	7.25	36.25
Easy-ID Low Voltage Cable, 24/2 Awg, 0.11" W X 0.06" thick, 12 VDC, 50' L	9697T1	McMaster-Carr	1	9.5	9.5
SS Compression Spring (outer diameter 1/4", 0.035" wire size, 2-1/2" length) 12/pk	9657K172	McMaster-Carr	1	3.8	3.8
Biocompatible heat sleeve 1/8" diameter, 4ft long (Alpha wire FIT heat shrinkable t	602-FIT3501/8-25	Mouser Electronics	1	7.39	7.39
Item Description - (Items Obtained Without Cost)					
Miniature Ergonomic Pinch Clamp (PP/Acetyl)	7,234,677	Z-Man Corporation	5	0	0
Clean, not sterile, Silicone tubing (0.58" x 0.077") Length = 50 ft	008490/000001	Specialty Manufactu	1	33.52	33.52
Sterile Monoject® 1mL TB Syringe 25 x 5⁄8" Detachable needle (box of 100)	07-806-1220	Webster Veterinary	5	0.17	0.85
Tissue Chamber Lids (PC sheet)	N/A	N/A	5	0	0
Cams (blue acrylic)	N/A	Higgins Labs	5	0	0
TOTAL					318.95
Remaining Budget					49.05